The EMAP Digital Atlas of Mouse Development

What is the EMAP Digital Atlas?

The EMAP Digital Atlas is an atlas of mouse embryonic development. It is based on the definitive books of mouse embryonic development by Theiler (1989) and Kaufman (1992) yet extends these studies by creating a series of interactive three-dimensional computer models of mouse embryos at successive stages of development with defined anatomical domains linked to a stage-by-stage ontology of anatomical names.

At least one representative 3D model embryo for every Theiler stage (TS) from TS07 to TS20 and one for TS26 is included in the Atlas, together with a comprehensive list of anatomical structures at all Theiler stages. A guide to Theiler staging of mouse embryos is also included.

The EMAP Atlas has been developed in a collaborative effort between the MRC Human Genetics Unit, Edinburgh and the Section of Biomedical Sciences, University of Edinburgh.

Theiler Staging Mouse Embryos

The general staging system chosen as the basis for the EMAP Atlas stages is that described by Theiler in "The House Mouse: Atlas of Mouse Development" (1989). This is used as opposed to days *post coitum* (dpc) or embryonic day (E) values as gestation times and rates of whole embryo or organ development can vary between different mouse strains. Theiler's definition of 28 stages of embryonic development are however too broad to distinguish some of the important phases of early development.

The EMAP Atlas therefore uses these Theiler stages as a framework for embryo staging but has also incorporated other staging criteria including somite number or those characteristics described by Downs & Davies (1993) to subdivide some of the Theiler stages into smaller increments. When present, these incremental stages are denoted by a letter, which follows the Theiler stage number (For example, in the EMAP Atlas, Theiler stage 11 is divided into stages 11a, 11b, 11c and 11d). Strictly though, the stages described by Downs and Davies apply to outbred PO mice. All other data in the staging pages and the EMAP embryo models correspond to embryos of crosses between F1 hybrid (C57BL6 x CBA) mice.

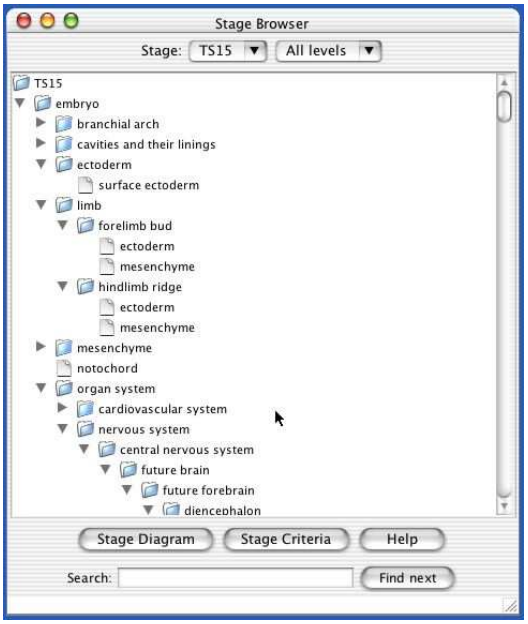
In the EMAP Atlas, each Theiler stage (or subdivision thereof) is linked to a corresponding diagram, with more details of the defining features for that stage. A brief text or pictorial index to the diagrams is also provided. The stages for which there is an EMAP model and links to view the model are indicated. See Appendix I for a printed version of the EMAP Theiler staging guide.

Main underlying components of the EMAP Atlas

Text Based Anatomy

For each Theiler stage represented in the EMAP Atlas, a standardised nomenclature for the histologically distinguishable anatomical parts has been developed. This information is organised in an hierarchical manner for each developmental stage and the nomenclature will be extended in the future to include information on cell lineage and cell type etc. The purpose of this nomenclature or 'ontology' is to provide a standardised vocabulary for mouse embryo anatomy as a framework for describing patterns of gene expression and other processes occurring during normal and abberant embryonic development.

An example of the content and structure of the anatomy ontology at TS15:



Naming Conventions and Data Formatting in the Anatomical Nomenclature

The nomenclature is separated for each Theiler stage and, currently, organised in a hierarchical manner such that complex structures are broken down on lower levels into their constituent parts.

All names are written in singular (e.g. 'somite' is used as opposed to 'somites') to simplify text searching and currently where there are obvious right/left duplications (e.g. limbs, somites, ganglia, etc.) only one name is given for the two structures. This will be modified in later versions to allow text annotation of left/right asymmetry.

Associated with some of the tissue names (to be extended) are synonyms. It is possible to use these names as alternative terms for querying the database.

Temporal Organisation and Groupings of the Anatomy Terms

Separate lists of terms for the anatomical components present at every Theiler Stage from TS01 to TS26 have been compiled. Each list includes all those components that are present during that stage and includes structures that differentiate into other structures during the stage. For example, both the otic placode and otic pit are included at TS14 because the otic placode develops into the otic pit during this stage.

Similarly, where different phases of development of a component are represented in space (for example, along the anterior-posterior axis) the names representing these different phases are listed under the same Theiler stage.

Each component is listed from the earliest stage when it can be reasonably identified morphologically, even in cases where the definitive morphology becomes clear at a later stage. A few structures (e.g. within the brain) have been named according to their future identity in order to facilitate regional descriptions without intending to imply precise demarcation.

Extra-embryonic membranes are not included in the Anatomical Nomenclature after TS12.

Spatial organisation and grouping of Anatomy Terms

At each Theiler Stage of development, the list is organised as a spatial hierarchy. The hierarchy starts with the embryo vs. extra-embryonic tissue and ends with individual tissues.

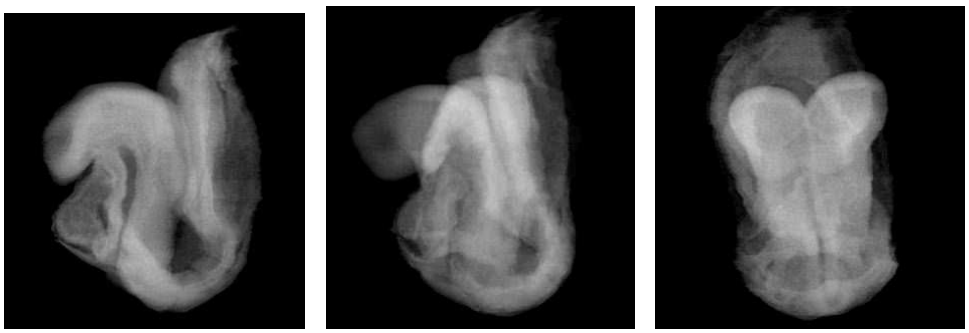
Some components are grouped under "organ systems" in order to provide a more convenient view of the data. The spatial hierarchy currently adopted is not a lineage hierarchy though some of the terms have lineage implications (e.g. ectoderm, mesoderm, etc).

Within each "branch" of the tree, components at the same level of the hierarchy are organised alpha-numerically. Although this can lead to difficulties in presenting the order of some components in space (e.g. the order of foregut, hindgut and midgut is presented in alphabetical order rather than from anterior to posterior), similar problems arise with any ordering system. The advantage of the alpha-numeric system is that users can always find a specific component according to the alpha-numeric rule.

We are developing alternative views of the list (i.e. different ways of grouping the components - for example, by tissue type or organ systems) and to provide the user with the means to create their own views. These new groupings will include information on the derivation of one component from another and will enable the user to follow the "lineage" of components to the extent that reliable information is available.

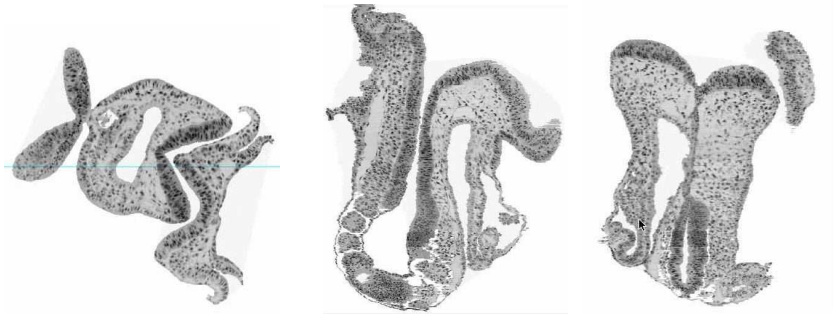
Structure Based Anatomy

For every Theiler stage between TS07 and TS20 and also for TS26, a 3D computer model of at least one representative embryo of that stage has been developed by EMAP. These models can be rotated in 3D space to allow visualisation of surfaces:



TS12 model rotating in space.

The embryo models can also be virtually sectioned in any plane to allow visualisation of internal anatomical structures:



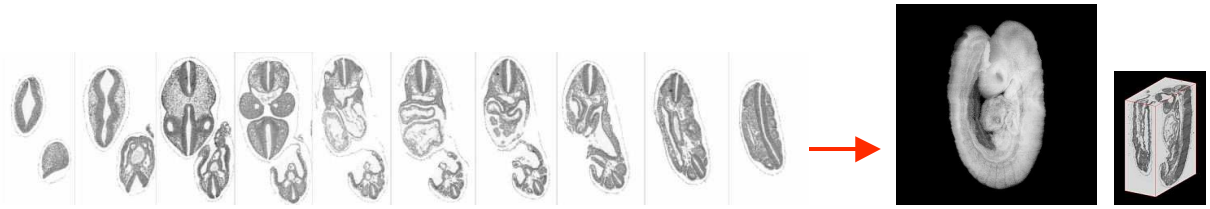
Virtual sections transverse, sagittal and frontal taken through the TS12 embryo model shown above.

Construction of the EMAP embryo models

Reconstruction from serial sections

Black and white digital images of entire serial sets of transverse histological sections (generally from the same embryos as illustrated in "The Atlas of Mouse Development", Kaufman (1992)) have been virtually stacked and aligned to create the grey-scale 3D models for embryos ranging from TS07 to TS14 plus the models for TS20 and TS26.

In this process, pixels from the 2D section images can be thought of as being transposed to become 'volumetric pixels' or voxels in the 3D model:



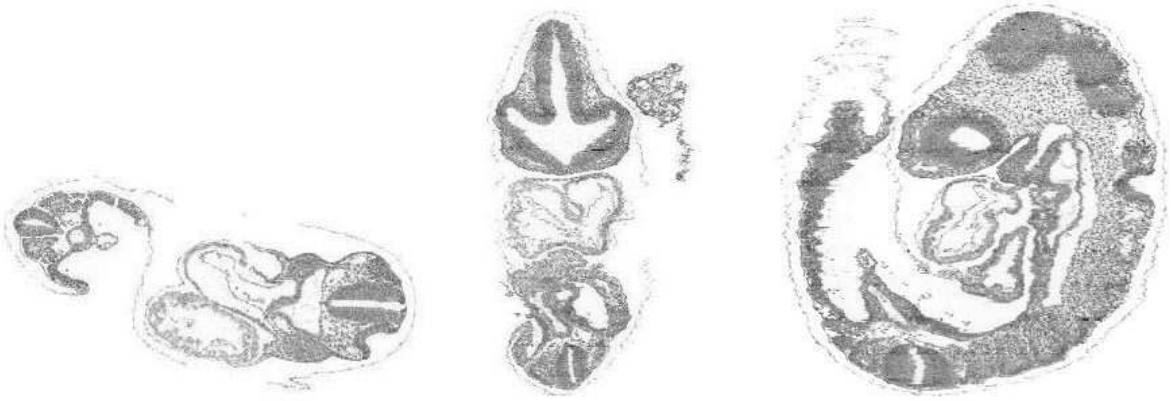
Series of digital images of transverse sections through one TS14 embryo

TS14 3D Reconstruction and a virtual block cut from it

The TS07-TS12 embryo specimens were embedded in plastic and 2 μm histology sections taken. Digital images of these sections were taken at a pixel resolution of 0.68 x 0.68 μm and sub-sampled by a factor of 3 to make the final voxel resolution of the models 2.04 x 2.04 x 2 μm. The TS13, TS14, TS20 and TS26 embryo specimens were embedded in wax and 7 μm histology sections taken. These sections were digitised at a pixel resolution of 1.36 x 1.36 μm and sub-sampled by a factor of 3 to give a final voxel resolution of 4.2 x 4.2 x 7 μm.

Artefacts introduced by damaged tissue sections were dealt with on a case-by-case basis. Comprehensive information on the artefacts and how they have been delineated is available in association with each model.

As each voxel in an embryo model is defined by a grey-scale value and its position within space along three axes, it is possible to 'cut' virtual sections of the embryo in any plane to reveal histological detail in that plane:

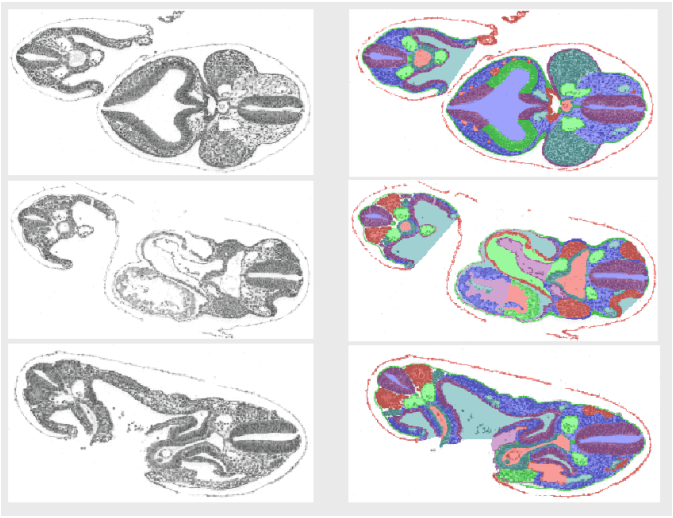


Virtual transverse, frontal and sagittal sections taken through the reconstructed TS14 embryo model

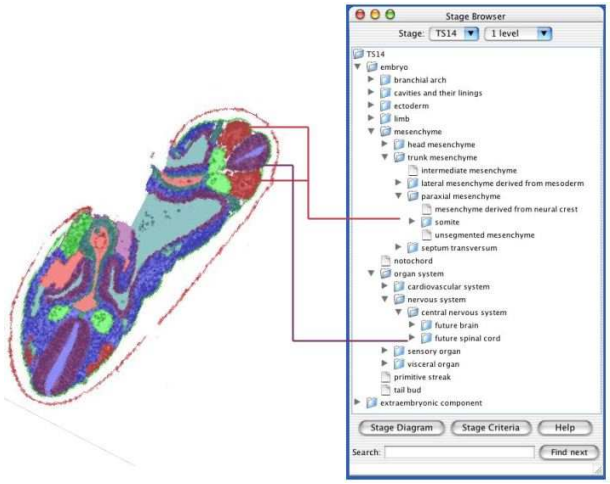
The models generated by reconstruction from serial sections have anatomical structures defined in three dimensions. This has been achieved by going through all of the virtual sections taken from each reconstructed model in one plane (generally in the

same plane as the original histology sectioning) and manually 'painting' each anatomical domain on every virtual section.

When a 2D domain is painted on each virtual section, the constituent voxels are grouped together and defined as belonging to a particular structure (e.g. neural tube or somites etc).

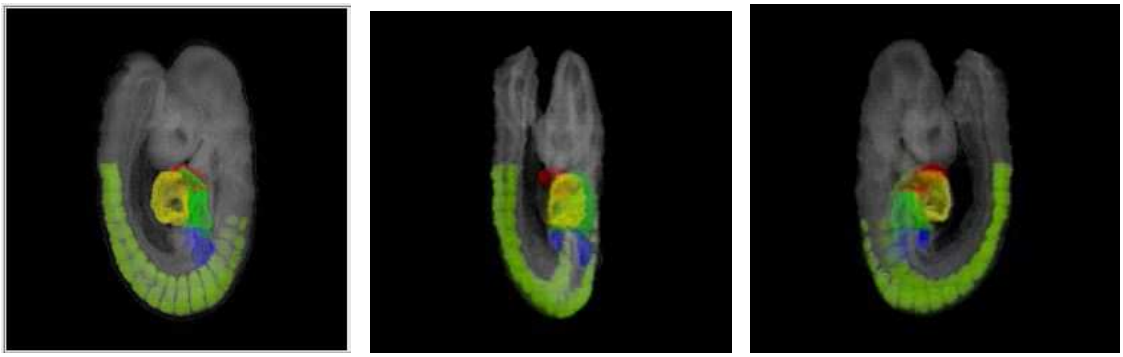


Example of 'painted' anatomy domains on the TS14 virtual sections



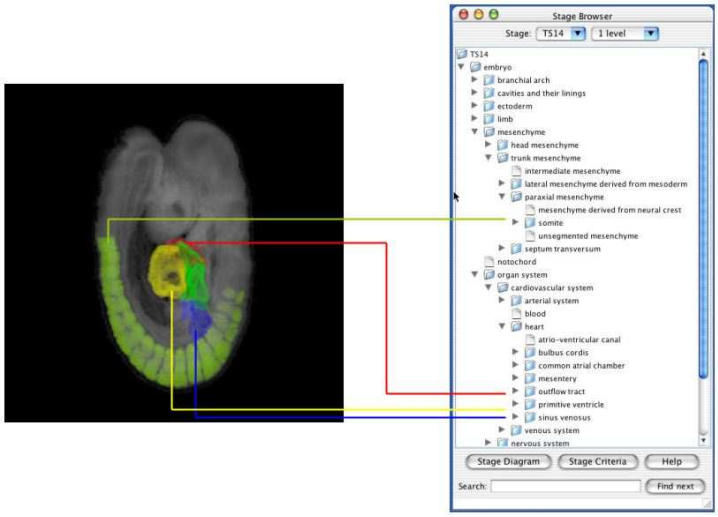
Painted areas on each section are then linked to the text anatomy descriptions

When every section has been completely painted, the anatomical structures are defined in 3D by their constituent voxels. These can be visualised as 3D views of the embryo model.



TS14 embryo model rotating in space showing 3D domains for somites (green) and the outflow tract (red), ventricle (yellow), atrium (green) and sinus venosus (blue) of the heart.

Anatomical structures defined in 3D space within the embryo models using this method are therefore also linked to the appropriate terms within the anatomy nomenclature:



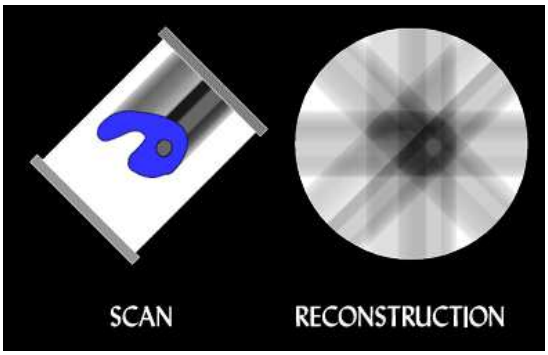
Areas within the 3D model are linked to the text anatomy descriptions.

Some components listed in the anatomy trees are not painted into the EMAP models because they are too small or have a stochastic "salt and pepper" distribution (for example, neural crest-derived cells that are mixed with mesodermally-derived mesenchyme in the 1st branchial arch). In these cases, they are included as part of a larger painted domain (for example, 1st arch mesenchyme).

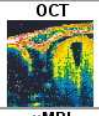
The Theiler stage models currently in the EMAP Atlas that have been generated by reconstruction from serial sections are TS07-TS14, TS20 and TS26.

Reconstruction using Optical Projection Tomography

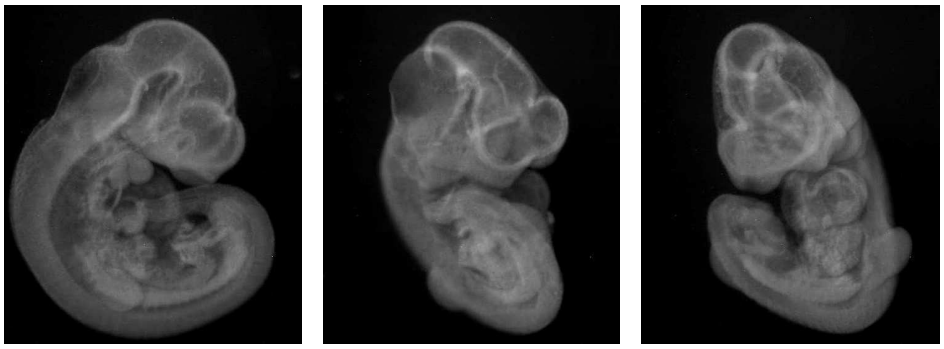
Optical Projection Tomography (OPT) has been developed at EMAP and used to construct further 3D mouse embryo models. This method relies on passing visible light through an embryo, which has been rendered semi-transparent by chemical treatment, and then capturing the projected image. These images record how much light passes through the specimen, so that darker regions indicate thicker and/or darker tissues. The embryo is rotated through 360° in small increments and a projection image captured for each point. Following one revolution, the whole set of digitised projection images are used to create a 3D reconstruction (Sharpe et al, 2002).



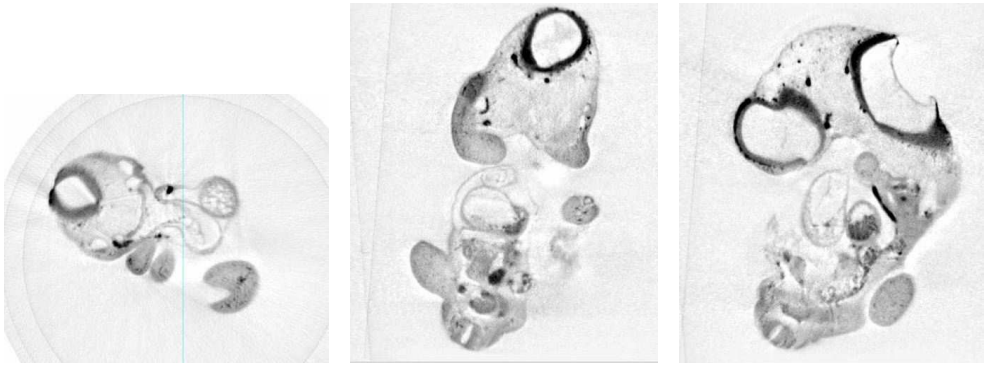
Voxel resolution in embryo models generated by OPT is approximately 10 x 10 x10 □m.
As well as being used to visualise unstained biological specimens in 3D, OPT can also be used to detect patterns of colorimetric or fluorescent staining.
The table below compares the OPT technique with other 3D visualisation methods. For further information on OPT, visit http://genex.hgu.mrc.ac.uk/OPT_Microscopy/optwebsite/frontpage/index.htm

Technique:	Advantages of OPT:	Disadvantages of OPT:
Confocal 	• can image bigger specimen • can image non fluorescent specimen, such as BCIP/NBT stained in-situs, or LacZ staining • cheaper	• cannot image live specimen • lower resolution
OCT 	• can image bigger specimen • can image commonly-used coloured or fluorescent dyes • cheaper	• cannot image live specimen
µMRI 	• can image smaller specimen with higher resolution • faster • much cheaper!	• cannot image live specimen • lower contrast for unstained tissue
Serial sections 	• faster • retains original 3D shape	• lower resolution

The EMAP models generated by OPT can be rotated in 3D space to allow visualisation of surfaces and similar to the embryo models generated by aligning serial sections, the OPT generated 3D models also have every voxel defined in grey scale and along three axes. As such it is also possible to 'cut' virtual sections in any plane through these models to reveal internal anatomical detail at cellular resolution::



TS17 model rotating in 3D space.



Virtual transverse, frontal and sagittal sections taken through the TS17 embryo model shown in 3D above.

OPT generated EMAP embryo models do not have any anatomical domains delineated in 3D space although these are currently under development and full text based anatomical ontologies have been developed for these stages..

The Theiler stage models currently in the EMAP Atlas that have been generated by OPT range include all stages between and including TS15 and TS19.

Resources for using the EMAP Atlas

The EMAP Mouse Atlas is available free online at http://genex.hgu.mrc.ac.uk.

Several Resources are available on-line as part of the EMAP Atlas which have been designed to allow the user to interact with the 3D virtual embryo models. The 'Section Browser' is pivotal to these interfaces and is described in Tutorial 1 below. The other on-line resources: 'Embryo View', 'Section movies' and the original sections used to make (some of) the models are also briefly described below.

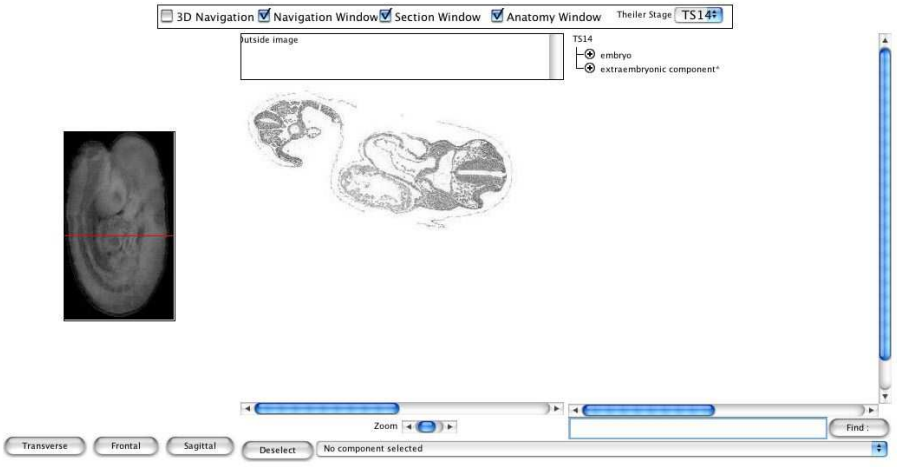
The EMAP Atlas also has an accompanying CD-ROM. The CD contains an extra program (MAPaint) that allows you to take virtual sections from the 3D models in ANY plane. MAPaint only runs on a UNIX operating system (eg. MacOSX, Linux, Sun/Solaris) and is described iin Appendix II. You can obtain a free copy the CD by emailing a request to:
ma-edit@hgu.mrc.ac.uk

The Section Browser

The Section Browser is the most powerful of the on-line interfaces for the EMAP Atlas as it allows you to view frontal, sagittal or transverse virtual sections from the 3D EMAP embryo models and for TS07-TS14,TS20 and TS26 (ie. those reconstructed from serially sectioned embryos and with delineated anatomical domains), you can identify the names of tissues within the displayed sections using your cursor. You can also find the location of a tissue within a section if you know its name. A direct link to the Jackson Laboratory's GXD database will also allow you to access a textual database with information on the genes expressed in a chosen tissue.

Tutorial 1: Using the Section Browser

- Go to the EMAP homepage at http://genex.hgu.mrc.ac.uk and follow the link to "3D embryo anatomy atlas", which will take you to a montage of the 3D EMAP embryo models available. Click on the model for **TS14**. The Section Browser will open, displaying three panels:



The left hand panel contains a volume rendered image of the embryo model with a fine red line showing the section plane that has been chosen. Smaller red lines at the side indicate the positions of pre-defined sections for selection.

- Move the red line between section increments and look at the changes in the central panel. Select any section plane towards the bottom of the model, and move the cursor across the display. You should see the different anatomical regions highlighted in blue. The name of the highlighted region will also be visible in the text box at the top of this panel.

  Click once on one of the highlighted regions and the name of this anatomical structure will be highlighted in red in the right hand panel. This panel represents information from the anatomical nomenclature database for the selected Theiler stage. The names marked with an asterisk indicate what structures are visible in the central panel display.

  Scroll down the nomenclature tree looking for any other names with an asterisk. Click on one of these names marked with an asterisk and the corresponding structure in the section plane will be highlighted in the central panel.

The nomenclature tree can be searched for structure names. Branches of the tree may also be exploded to reveal further structures.

- Type the term ***eye*** into the text field below the right hand panel, and hit "find". Explode (open) both the optic eminence and optic vesicle branches. You should see that there are no eye structures visible on this display, as nothing is marked with an asterisk. This is not surprising, as the section does not pass through the eye!

  Move the red line so it rests on the fifth increment from the top. This is a section view through the eye of the embryo. Once again, type ***eye*** into the right hand text field and hit "find". You should now notice that "optic vesicle" is now marked with an asterisk, indicating its presence in the displayed section. Click once on this name and the structure will be highlighted in the section display.

The text search mechanism currently only supports simple text queries. For notes on the naming conventions used and tips for searching the anatomical terms database refer to the section at the start of these notes entitled 'Text Based Anatomy'.

The view of the whole embryo changes according to whether the transverse, frontal or sagittal plane of section has been chosen.

- Select the **frontal** section plane by clicking on the appropriate buttons benath the image of the whole embryo and see if you can idenitify the ***optic vesicles*** in this section plane.

- Now select the **sagittal** section plane and find the ***ectoderm of the 1st branchial arch***.

- Select **TS10** from the drop down menu above the right hand panel of the section browser. Select "3D navigation" by clicking in the box to the left hand side of the header bar of the section browser. This box should now be ticked to indicate this option has been selected. The section plane of the embryo will represent a transverse section.

  Click anywhere on the red line of the display. As you move the mouse up and down, you will change the plane, as demonstrated in the earlier exercise. As the mouse is moved left or right, the display of the embryo model will rotate.

It should be noted, that 3D navigation is only possible when the transverse option is selected.

- De-select "3D Navigation" and click once on the "frontal" button. You will see that the red bar is now vertical and the image in the central panel has changed to show the frontal section plane.

  Click on the "sagittal" button. This time the image in both panels will change, to represent the section plane through the embryo and resultant section.

  Move the red line indicating the section plane to the middle section and select from the bottom right hand menu on the section browser "embryo.primitive streak". This should now be highlighted on the section display in blue, and in the nomenclature tree in red.

This menu contains all the structures that are marked with an asterisk in the nomenclature tree, i.e. all those visible in the current section display.

- To remove the highlighting on the display, click the “deselect” button below the central panel.

Each display in the central panel may be re-sized by using the zoom bar. The images may show increased pixelation at very high resolution.

- Select TS12 and browse across the section for “Future Brain”. Highlight the section by clicking once on it with the left hand mouse button. Now place your mouse over one of the highlighted sections, and click with your right hand mouse button (if using a Mac use the apple key while pressing the mouse button). A floating link will pop up to the gene expression database (GXD) at the Jackson Laboratory.

Select this link to view the information contained in the Jackson GXD on the genes expressed in the future brain (see the “Structure” column). This information will pop up in a separate window. Note that there is no spatial information to the exact location of gene expression within the structure, however, the particular areas of the brain are indicated by the text description.

What are the first 10 genes held in the GXD in the future brain at TS12?_____

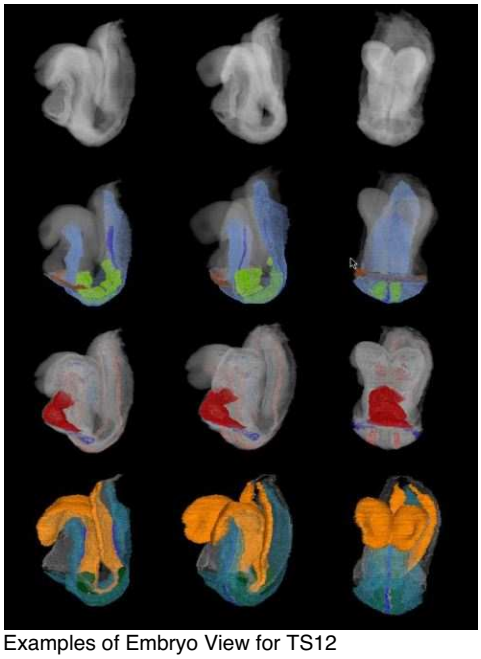
Other on-line EMAP Atlas Resources

All of the following resources are available on-line by clicking on the "More Theiler (stage #) Resources" panel on the bottom of the Section Browser page described above.

EmbryoView

EmbryoView allows you to interactively view the 3D embryo model from various angles. Also included is the option of simply playing mpeg movies of the embryo model rotating in space.

Embryo models ranging from TS07 to TS14 (i.e. those reconstructed from serial sections containing defined anatomical domains) can be viewed with or without various pre-selected 3D anatomical domain highlighting.



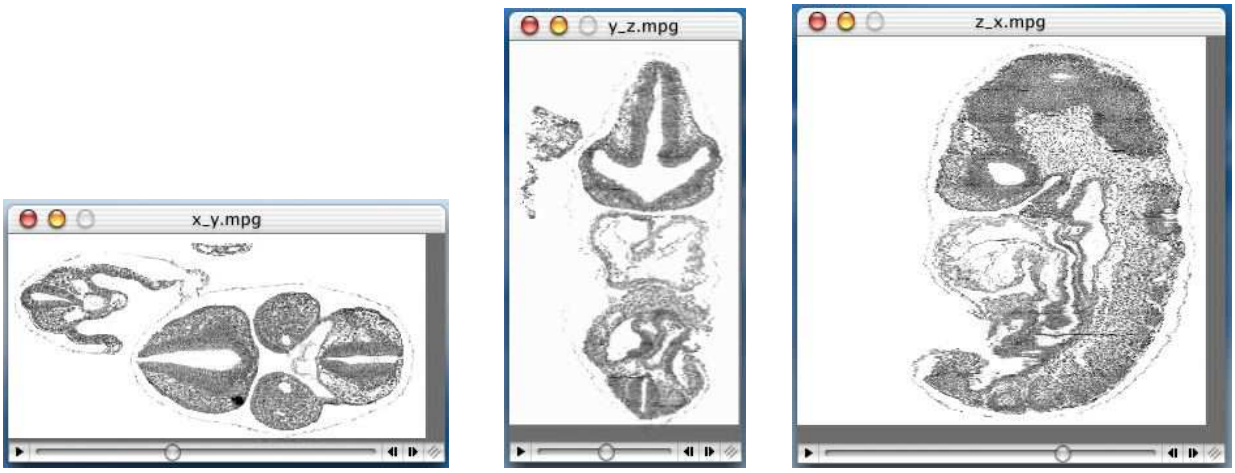
Examples of Embryo View for TS12

Embryo models ranging from TS15 to TS19 (i.e. those reconstructed by OPT) do not have defined anatomical domains within them and cannot be viewed with anatomical regions highlighted.

You need a Java enabled Web Browser to use Embryo View. See Appendix III for suggested Web Browsers to use with your computer's operating system if you have problems.

Section Movies

These are collections of all of the virtual transverse, sagittal and frontal sections that can be taken through each embryo model. They are presented as mpeg movies and to view them, a movie player must be installed on your computer[1].

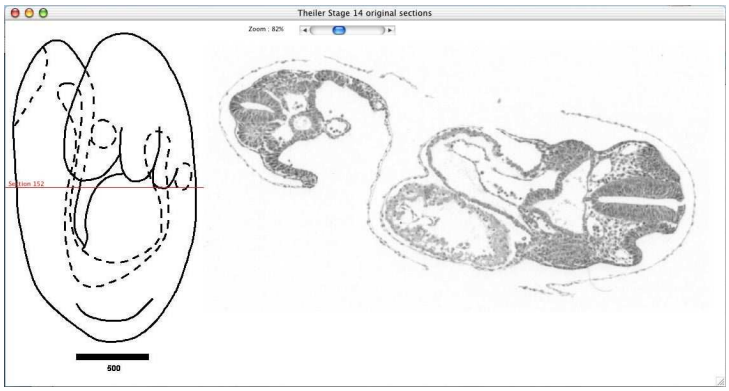


Section movies of TS14

They differ to the Section Browser in that every section within that plane are presented from one side of the embryo to the other and they are not interactive (i.e. you cannot identify the name of structures within them).

High Resolution Section Images

These are high-resolution digital scans of the original transverse histological sections that were used to construct the TS07-TS14, TS20 and TS26 embryo models. The sections are numbered and you can view any section image from the set. There are no original sections for the TS15-19 embryo models as these have been constructed using OPT.



[1] This will be the case on the majority of machines, but if there is nothing installed on your machine, there are plenty of free options available.

You need a Java enabled Web Browser to view the Original Sections. See Appendix III for suggested web browsers to use with your computer's operating system if you have problems.

Note: For the TS7, 8, 11 and 12 embryos, histological sections were taken from the base of the embryo whereas for the TS9, 10, 13, 14, 20 and 26 embryos histological sections were taken from the top. This means that the original sections on the microscope slides and the corresponding digitised section images are effectively viewed from opposite sides for the two groups.
There are no original sections for the TS15-19 embryo models as these have been constrcuted using OPT.

The EMAGE gene expression database

EMAGE (Edinburgh Mouse Atlas of Gene Expression) is a publicly available database of gene expression patterns during mouse embryo development.

Expression patterns held in EMAGE are denoted in space by domains mapped to the standard set of EMAP virtual mouse embryos at different stages of development. The data is also described using a set of standardised text descriptions for sites of expression, the pattern of expression and signal strength. The EMAP Digital Atlas of Mouse Development acts as the standardised housing framework.

EMAGE is part of the Mouse Gene Expression Information Resource (MGEIR) which also includes the text based Gene Expression Database (GXD) that has been developed and is maintained by the Jackson Laboratory, USA. GXD stores primary data about endogenous gene expression from different types of assays (e.g. gels, blots and *in situ* analyses of mRNA and protein distribution) in different mouse strains and mutants. GXD records are generally extracted from the literature by a team of scientific curators and indexed as text based records complemented with digitised images of the original published data. GXD places gene expression data in the larger biological context by links with other resources

The data in EMAGE primarily comes from direct submission by researchers. These are curated by the EMAGE editorial office prior to inclusion in the database. EMAGE editorial staff also spatially map gene expression patterns that have previously been extracted from the literature and indexed using text descriptions by the Jackson Laboratory's GXD curatorial staff.

One EMAGE entry is the pattern of gene expression detected using one probe at one Theiler stage of development.

EMAGE has been developed and is maintained at the MRC Human Genetics Unit, Edinburgh.

Text based data in EMAGE

Information stored as text in EMAGE includes:

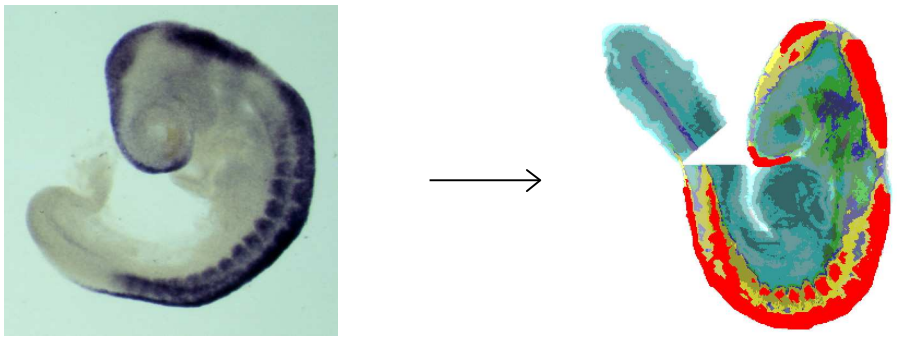
- Names and contact details of the submitting authors and principal investigator.
- The name and symbol of the gene that is being studied.
- Details to uniquely identify the probe (name and nucleotide sequence of probe, origin of the clone used to generate the probe etc).
- Details of the specimen (developmental stage, strain, sex, fixation method etc)
- Annotation of the expression pattern to the text-based EMAP anatomical terms.
- An EMAGE accession number to identify the entry.

Spatial data in EMAGE

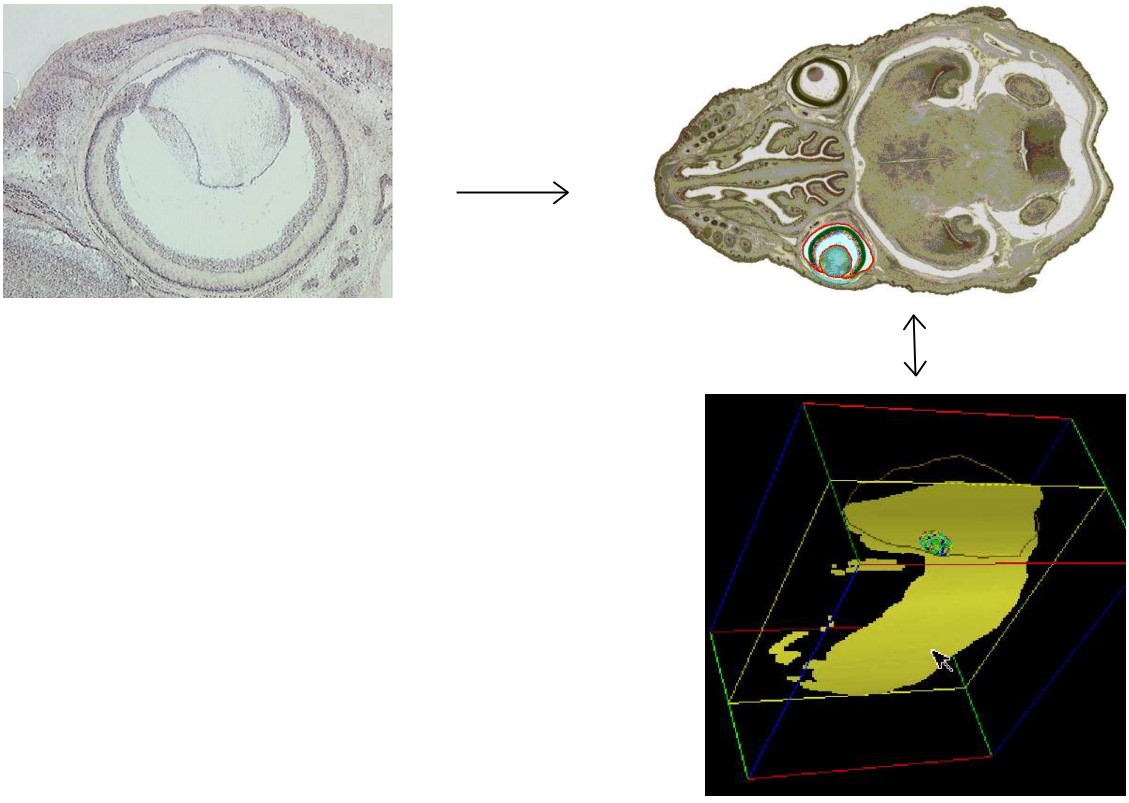
Spatial information stored in EMAGE includes:

- Images of the original data. These can take the form of digitised photographs of 2D expression data or raw OPT 3D data.
- Spatially mapped data - This contains domains corresponding to areas of gene expression that have been mapped into the space of the standard framework models.

Expression data from photos of stained whole mount embryos is extracted and transferred onto a corresponding surface view (left or right) of the age-matched standard 3D embryo model. Different colours in the model depict different expression levels of the gene and it is these mapped images that underlie returns from the central database:



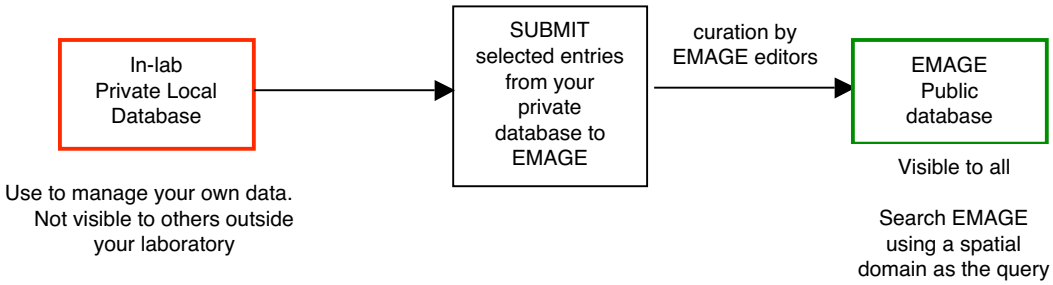
Expression data from images of histology sections or OPT imaged embryos is extracted and transferred onto corresponding sections that have been selected within the age-matched 3D embryo model. Data in this case is mapped into individual voxels *within* the 3D model. As above, the different colours represent different expression levels (brown represents regions not examined in this experiment).



EMAGE database structure

The EMAGE database and accompanying software has been developed to allow you to use the same package to search the central EMAGE database, make your own private local databases for in-lab data management or submit gene expression data to the public EMAGE database.

Local databases emulate all the functions of the public database, with the exception of spatial queries, which can only be used in the public resource.



The databases are written using the Java programming language. To use them on your computer, you must have Java Web Start installed. Your computer also needs to be Java compliant, with Java 1.4 (JRE2 v1.4 (Java Runtime Environment 2) or above on it[1]. For the purposes of this course, Java will have been installed on your machines. To do this in your home laboratory, please see the instructions on the EMAGE download webpage.

[1] For the majority of computers, Java will have been installed when it was set up. For older computers, it may be advisable to check with your systems administration whether version 1.4 is available.

Tutorial 2: Searching the Public EMAGE Database

The interface to this database has been written in Java, and as such, certain components must be downloaded to your computer before you may search its contents.

- Go to the EMAP homepage at http://genex.hgu.mrc.ac.uk/ and select “EMAGE gene expression database”. On the EMAGE introduction page, follow the “Download EMAGE start file” link.

  Register for use of the database by entering your name and email address in the appropriate fields and click on the “Continue” button. The web browser will take you to the appropriate download page for your computer’s operating system. In this case it will be the UNIX download. Instructions for PCs and Mac OS X[1] will be found on the appropriate webpage when performing the download.

Ignore step one on this page as it contains links to the instructions for downloading both Java 1.4 (or JRE2 v1.4 (Java Runtime Environment 2)) and Java Web Start as these are currently installed on the computer you are using. You may, however, need to follow these instructions when installing in your home laboratory.

- Select “Download EMAGE software” and save it on your hard disk (this may be done automatically). The necessary files will be downloaded to your computer. You can monitor this progress in the application window visible on this screen. After a small delay, the EMAGE window will pop up containing a smaller, **Query** window. Should this Query window not be open, select the “Central DB” menu and then the “Get by Query” option.

The top bar on this query window is formulated as a question, with drop down menus allowing you to select the individual options that will ultimately make up your query. There are therefore four questions which can be used to interrogate the database:

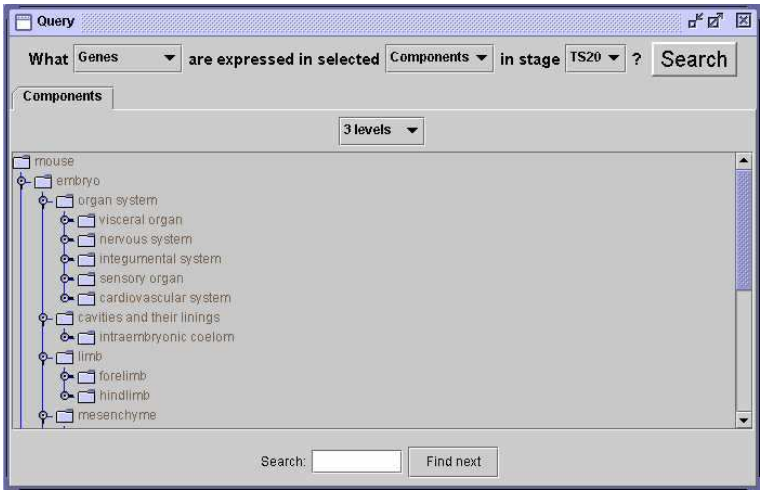
What genes are expressed in selected components at a particular stage of development?
What genes are expressed in selected regions at a particular stage of development?
What regions express listed genes at a particular stage of development?
What components express listed genes at a particular stage of development?

[1] There is no download for Mac OS9 or below.

What *GENES* are expressed in selected *COMPONENTS* in stage *X*?

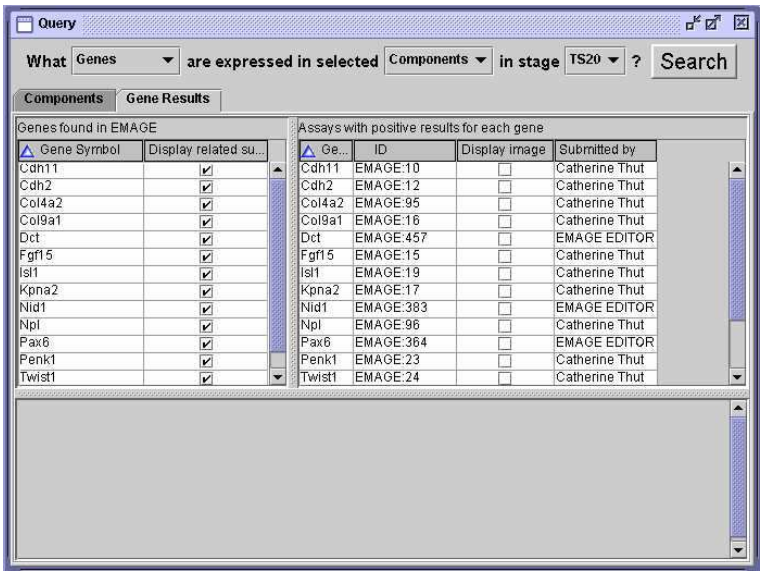
This query is used to find the genes expressed in an anatomical structure you know the name of.

- Select TS20 from the drop down menu in the query. The Anatomy ontology for TS20 will be loaded as such:



- Now, type ***eye*** into the search field at the bottom of the window, and hit “Find Next”. Eye will be highlighted in the nomenclature list by a white box.

- Mark the ***eye*** component by clicking on it once with the right hand mouse button[1]. The component name will turn red. Now hit the “Search” button in the top right hand corner, which will return the results of the search in tabular format as below:



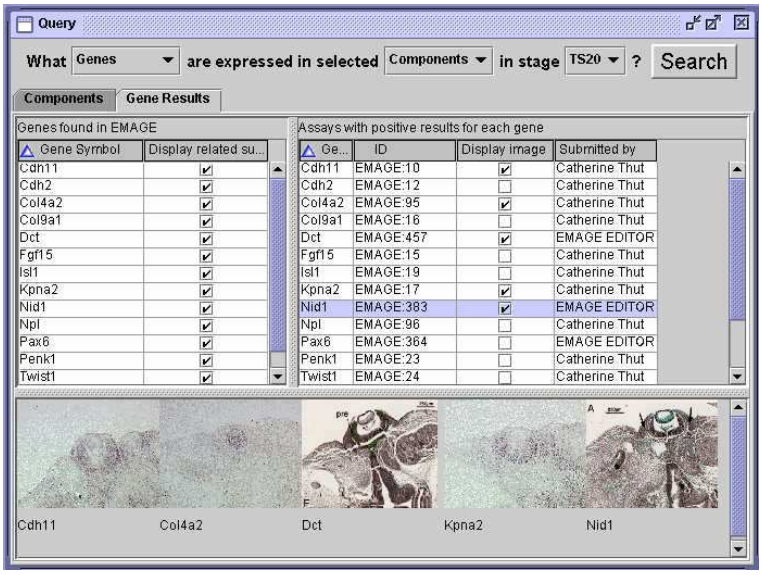
The tabulated results appear in the same query window as a separate tabbed pane called “Gene Results”. On the left hand side is a list of the genes found in the EMAGE database using the initial query. All those that are selected (i.e. have a tick in the box to the right of them) will appear in the table to the right of the pane. This table includes the name of the submitter, and the EMAGE ID number.

What genes are found in the database that are expressed in the eye at TS20?

_____

[1] Mac users should click on the component name once, whilst pressing the “Apple” key.

- In the right hand table, select “Display Image” for Cdh11. This image will be loaded into the lower panel. Repeat this for a couple of other entries.



The images seen in the lower panel are the original images used in the data mapping for each submission. Both the gene name in the right hand panel, and the image in the lower panel are linked to further information in the database.

- Double click on the Cdh11 image in the lower panel and a new, tabbed window will pop up. This may be expanded by dragging the corner of the window. The borders are also movable.

The EMAGE ID number for the submission is visible in the top left hand corner of this window as well as the gene and the stage.

- Click on the “Person” tab to display the information of the submitter and principal investigator of the data.

  Who has submitted this data?_____

- Click on the “Probe” tab to display information on the probe used in the original experiment. You can see here, that this probe was generated from a clone of partially known DNA sequence.

  What is the name of the clone used to generate the probe used in this experiment?_____

  What is the corresponding (partial) sequence entry in EMBL/GenBank for this clone? _____

  How was this probe labelled? _____

- Click on “Specimen” tab to display information on the specimen used in the experiment. This information includes the Theiler stage of the embryo and an original data image.

  What strain of mouse is this data from?_____

  How many days post coitum was this specimen at harvesting?_____

- Click on “Expression Mapping” to display the original image again. Information is available on the spatial and textual mapping of this data.

The colours used to annotate expression are as follows: red = strong expression, yellow = moderate expression, blue = weak expression, green = possible expression, cyan = no expression detected, grey = not examined (unnanotated).

  In what anatomical components have the authors denoted expression?_____

  To what level of expression? _____

How many levels of expression intensity have been denoted by the authors in the spatially mapped image and what are they? _____

- ❑ Click on the “Links, Acknowledgments and References” tab to display this information. If a paper is relevant to the entry, it will be noted in the Reference List.

  What references are associated with this EMAGE entry?_____

- ❑ Click on the “Show in Browser” button. This will display the details of the paper in PubMed.

- ❑ Close this submission's window but NOT the query window

- ❑ Now select TS15 from the drop down menu in the query question to read - "What genes are expressed in selected components at TS15?"

- ❑ Type "mesencephalon" into the text field and then press the "Find next" button. You should notice a word in the list of anatomical terms highlighted in yellow. Terms marked in yellow in this manner represent synonyms.

  What is the alternate name of this structure? _____

- ❑ Select this term by clicking with the right hand mouse button (the lettering will change to red) and then hit "Search"

  What genes are expressed in this stucture at TS15? _____

What *GENES* are expressed in selected *REGIONS* in stage *X*?

There are two types of spatial searches you can perform here - searching **lateral views of whole mount embryos** or **searching within the 3D space of an embryo**. A single search can be selected by selecting the appropriate tab in the window.

Whole Mount Data

- Return to the Query window and formulate the following question at the top of the window: "What genes are expressed in selected regions at TS11?"
- Click on the “Whole Mount” tab if it is not already active.

There are two ways to specify the search domains for whole mount data, the first is called 'Paintbrush' and is available for all embryo stages from TS07-TS19 and involves painting a region of a left or right lateral view of a standard embryo to define the query domain.

The second option is called 'Predefined Region' and is currently only available for TS14 and TS15. This option involves moving the cursor across a left or right lateral view of the standard embryo model to highlight pre-set regions as the query domain.

Defining a search domain by painting a region

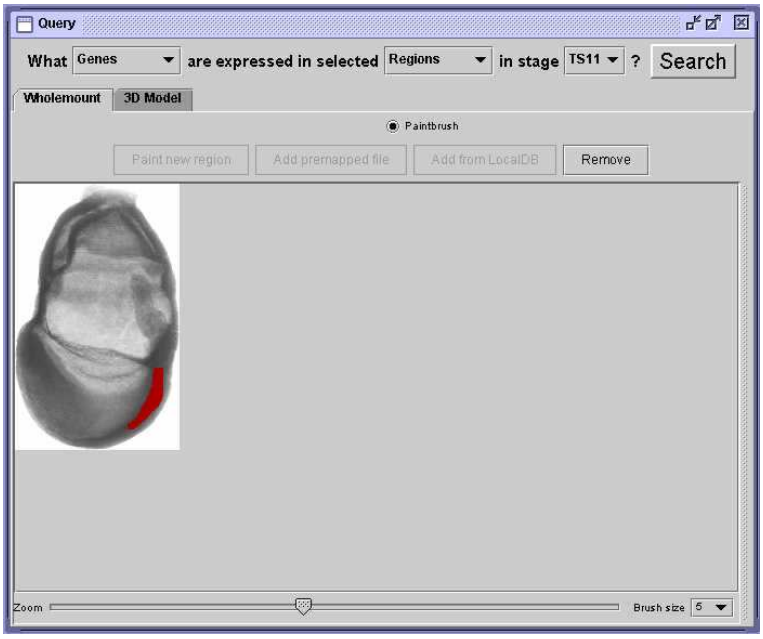
- Click on “Paint new Region” button. A “Select Wholemount View” window will appear.

Select the left or right view from the pull down menu at the top of the window and click on “Choose”[1]. The view you have selected will be loaded into the Query window and the cursor will appear as a cross.

For some embryo stages (eg. TS15), there are views of more than one embryo (they are at different time-points during the Theiler Stage - data sets have been mapped to either model and these are linked to each other to allow data returns of both using either model for the query).

- Paint the region shown in the figure below. The brush size can be changed using the options from the pull down “Brush Size” menu. To remove an area painted in error, click the “remove” button and to change the wholemount view from the to right, click on "remove" a second time.

[1] This is necessary to load the image into the query window as a result. Failure to do this means that the image remains provisional, and cannot be used independently.



- Hit “Search”. This will once again display a “Gene Results” tab (see above). Double click on ***EMAGE:6*** in this pane. This is an example of an entry, which has been previously indexed using text in the GXD and mapped and entered by the Editorial Office at EMAGE.

You should see the name of the original author, and note that this was mapped by EMAGE. This data was originally published in *Development* (this information can be displayed by clicking on the image on the “Specimen” tab) and was indexed by our collaborators at the GXD by annotating the EMAP anatomy ontology according to the original authors' description in the paper.

Where was this data originally published and by whom?_____

What is the description of the Fgf8 probe used in this study?_____

What are the three anatomical components the authors have defined as sites of expression?_____

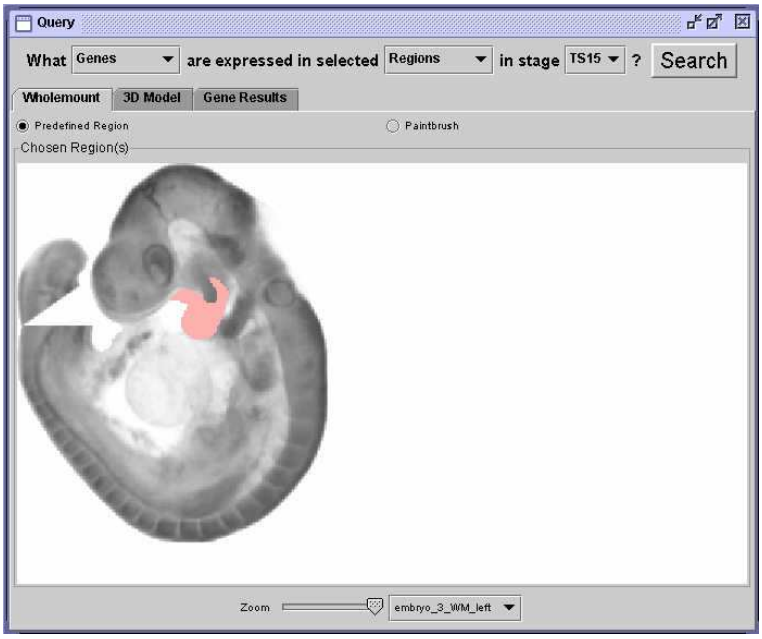
- Now select the “Links, Acknowledgements and References” tab and highlight the MGI identified entry (MGI:1335427) by clicking once on this information. Hit the “Show” button and the entry as it is held in the GXD will appear in a browser window.

- Close the submission window.

Predefined Search Regions (for TS14 and TS 15 embryos)

- Select TS15 from the list in the original query and select the “Whole mount” tab. This time, the 'Predefined Region' Option will be selected as the default with the left hand side of the embryo will be displayed as default. Should you wish to change this, you can do so using the drop down menu at the bottom of the image. Using the zoom bar will make the image larger or smaller.

- Move the cursor across the embryo view - different regions will be highlighted in yellow as the cursor moves across them. To define a region as the query domain, click once using the mouse button while the region is highlighted in yellow - it will change colour to pink. Highlight the region as shown in the figure below:



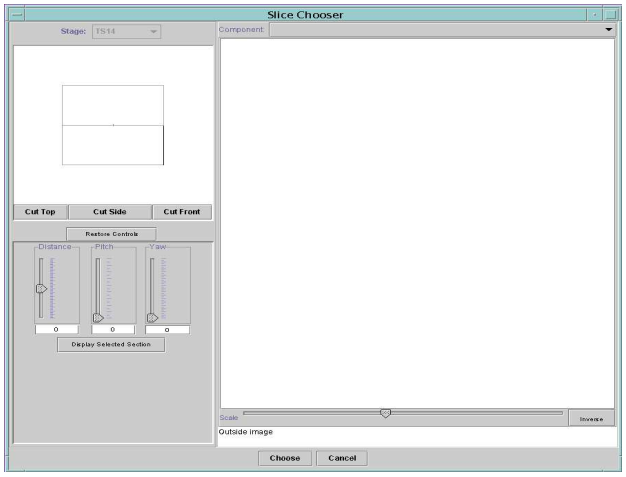
- Click on the “Search” button in the top right hand corner of the Query Window and the genes expressed in this region will be returned in the “Gene Results” pane.

Which genes are expressed in this region? _____

- Browse through these entries to see how the data was orginally spatially mapped and why they have been returned in this query.

Three Dimensional Data

- Select TS20 on the original query. There are no wholemount veiws currently avaibale for TS20 and only data housed in the 3D space of the virtual embryo model. Click on “paint new region” and the “Slice Chooser” will appear in a separate window.



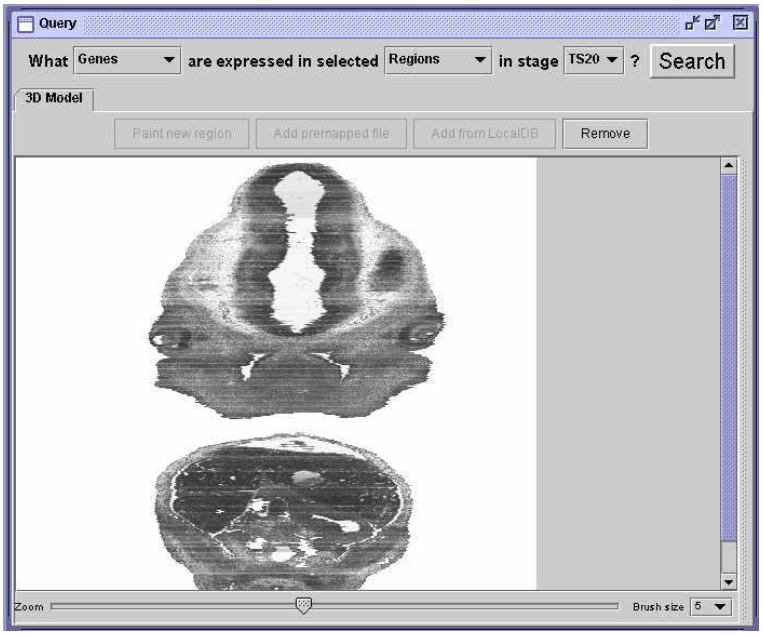
The square in the upper left hand panel is the front view of a box that represents the 3D space that the standard embryo model resides within. It can be rotated in 3D space using the cursor. The Distance, Pitch and Yaw sliders may be used

to select a section plane. Alternatively specific coordinates may be typed into the fields below the sliders to define a section plane.

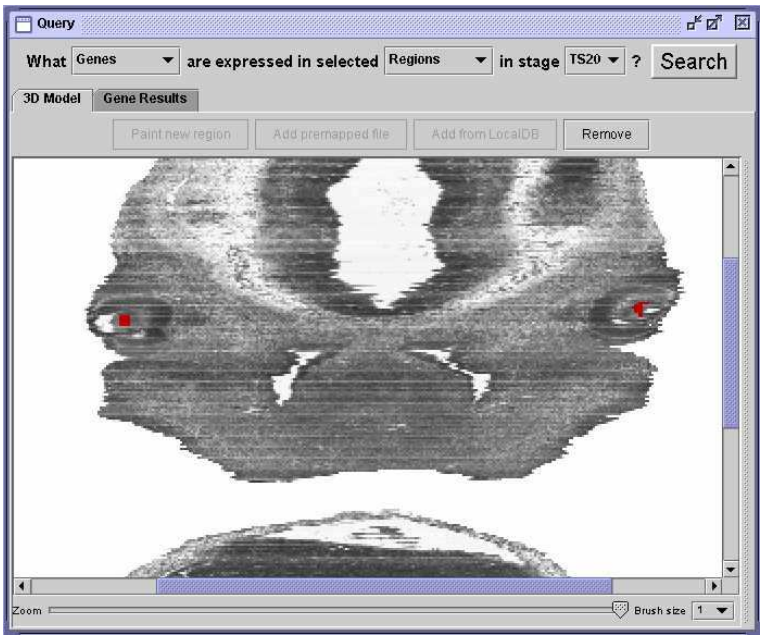
- Type "-66" in the Distance field and press return, "90" in the Pitch field and press return and ”0” in the Yaw field. Click on the “Display Selected Section” button. You MUST hit return after entering these numbers in the relevant fields, otherwise they will not be activated in your chosen section.

Click on the 'Choose' button at the bottom of the window.

The chosen section will be loaded into the Query window (this is a section through the eye) and the cursor will appear as a cross.



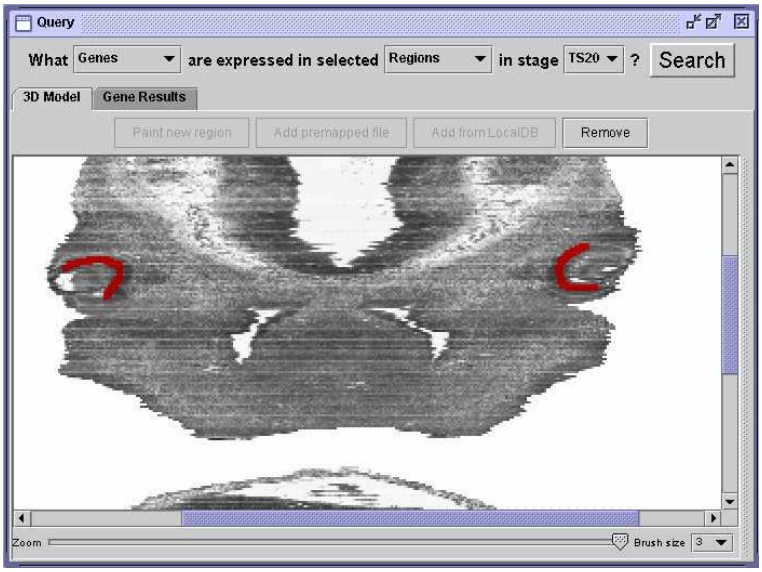
- Paint *only* the lens region as shown on the figure below. The brush size can be changed using the pull down “Brush Size” menu. Hit the “Search” button.



This will once again display a “Gene Results” tab (see above). Display the associated images - you will note that they are expressed in the lens. Peruse through some of these entries to see how the original data has been mapped using both spatial domains and as text.

What genes are expressed in the lens, as defined by this spatial search? _____

- ❑ Go back and perform another search, but this time, search a domain in the retina as such:



What genes are expressed in the retina, as defined by this spatial search?_____

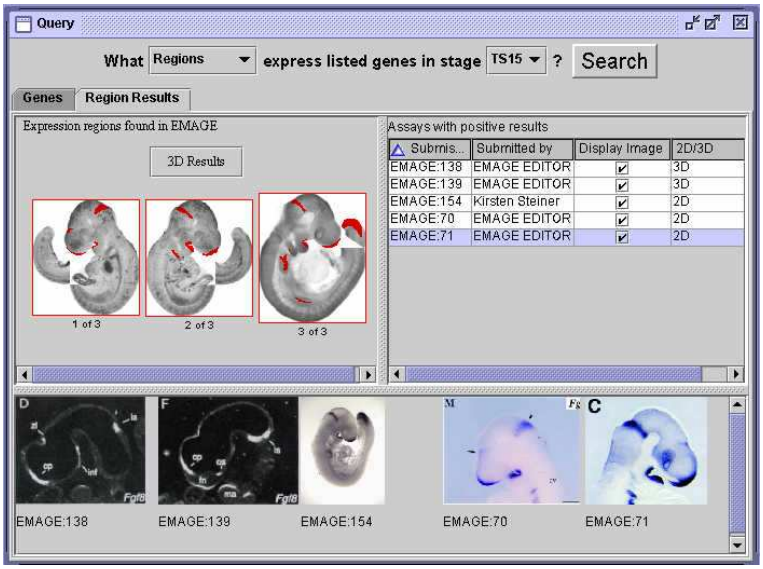
What *REGIONS* express listed *GENES* in stage *X*?

- ❑ Formulate this question and select TS15 and type **Fgf8** in the text field beside the Add button.

Data entered in EMAGE is done so using the Mouse Genome Nomenclature Committee approved gene names and symbols. The currently approved names can be found at http://www.informatics.jax.org - Please use these approved symbols for searching (case-insenstive).

- ❑ Click on the “Add” button. The gene symbol will be entered into the list below. Now hit “Search”.

The results are returned in the “Region Results” tab, which consists of two panes, the “Expression regions found in EMAGE” (left hand pane) and “Assays with positive results” (right hand pane) as below:



- ❑ Look at the list in the right hand pane and note that the entries EMAGE:138 and EMAGE:139 are described in the final column as 3D data. This means the data has been mapped into the 3D space of the embryo model, and not onto the lateral view of a whole mount. The other entries are denoted as 2D results (ie. mapped onto the surface of a wholemount virtual embryo and these mapped regions are shown on the left).

- ❑ Double click on the EMAGE:138 entry to display the information on the original gene expression experiment and mapped data.

In which structures has Fgf8 been annotated as being expressed in within this entry? _____
_____

These spatially mapped results can be saved in order to re-display them in MAPaint and look at the mapping in three dimensions. This can be done by clicking on the '3D Results' button. In upcoming versions of the EMAGE database it will be possible to automatically use single or collections of spatial domains obtained from the central database to perform further interrogation of the database.

What *COMPONENTS* express listed *GENES* in stage *X*?

- ❑ Select TS17, remove Fgf8 from the list of genes and then add **Notch1** to the list. Now, hit “Search”.

The results are returned in the “Component Results” tab, which consists of two panes, the “Expression found in EMAGE” (left hand pane) and “Assays with positive results for each component” (right hand pane).

You will notice that many structures have been annotated.

How many corresponding EMAGE entries are there for all of these named structures? _____

- ❑ Double click on EMAGE:243 to open this entry.

Which structures have been annotated as expressing this gene in this experiment?_____

- ❑ Within the EMAGE:243 entry, move the cursor over 'cardiovascular system' that will be highlighted in red. A pop-up box will appear with further information.

What does this extra information say for cardiovascular system in EMAGE:243?_____

- ❑ Now work your way up the tree and list all of the structures higher in the heirarchy (eg. organ system is higher in the tree than cardiovascular system).

List these 'higher order' structures for EMAGE:243_____

The names of these higher order structures are also returned from queries in EMAGE. This means that is necessary to only annotate the lowest point of the anatomy tree to return higher level terms. If you were to perform this process for all of the entries returned for this query (ie. EMAGE:242, 243, 244 and 245), the additive results would match what is displayed in the annotated list that has been returned from the central database.

Making a Local Database

After downloading the EMAGE software, users within a lab can create a private local database. Any number of local databases can be created - e.g. each member of a laboratory can create a database to manage their own data.

The software allows the user to store the name of the experimenter, the details of the probe and specimen, experimental notes, original data images, notes that are critical for interpretation of the data, and data that has been spatially mapped into the framework of the EMAP mouse atlas. This is the same type and format of information you have seen in the central database.

During data entry, the user assigns their own names (the "LocalID") for each entry. Searching your own local, private database allows only text searching on the query. None of the spatial information is searchable in this database. For this, the central database must be queried.

Full instructions for making a Local database, making entries within it and completing these entries can be found in the 'Help" menu within the EMAGE software application.

Submission to the public EMAGE database

Following data entry into your local database, any number of specific entries can then be submitted to the EMAGE Editorial Office for inclusion in the public database.

Registration is required for submitting data to the public EMAGE database and is used to protect access to the data associated with each submission.

Data received electronically over the web by EMAGE is automatically given a temporary ID number (the "TempID") and sent to the Editorial Office for curation. Following curation, an editor will contact the submitter to check any queries and to finalise the submission prior to deposition into the public EMAGE database.

An alternate way to submit data to the public EMAGE database is for researchers to send images (original photographs or image files) or samples (histological sections or whole embryos) directly to the EMAGE Editorial Office. Editors will assign a TempID number for the submission and contact the submitter with this number. Following digitising and mapping of the data by the editor, he/she will then contact the submitter to ensure that the mapping has been performed accurately. Once both parties are satisfied, the submission will be forwarded to the public EMAGE database.

When a submission is deposited in the public EMAGE database, the TempID number is replaced by a permanent EMAGE ID number ("EMAGE ID").

It is possible to organise a publication embargo date with an editor to ensure that data does not appear in EMAGE prior to scientific publication in a journal.

Information held in EMAGE is owned by the submitter.

APPENDIX I: Theiler Staging Guide

Theiler Stage 01

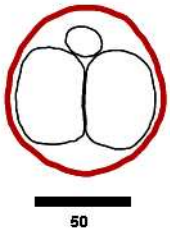
One-cell stage embryo (fertilised egg)
1 cell. Zona pellucida present.

0-0.9 dpc (range 0-2.5 dpc)
Scale bar 50μm

Theiler Stage 02

Dividing egg stage
2-4 cells. Zona pellucida present. First cleavage occurs at about 24 hours.

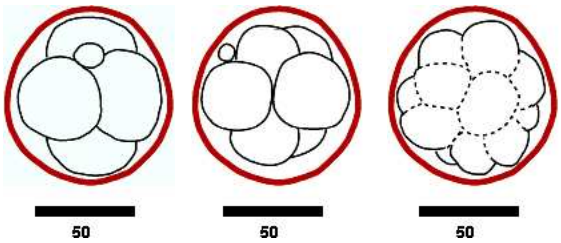
Embryonic age = 1 dpc (range 1-2.5 dpc)
Scale bar 50μm



Theiler Stage 03

Morula (early to fully compacted)
4-16 cells. Zona pellucida present. Usually found in the oviduct towards the utero-tubal junction.

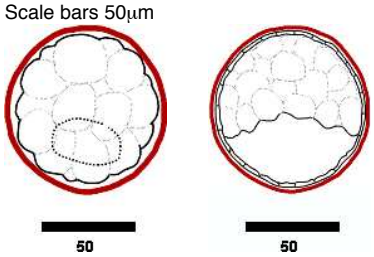
Embryonic age = 2 dpc (range 1-3.5 dpc)
Scale bars 50μm



Theiler Stage 04

Blastocyst (ICM apparent)
16-40 compacted cells. Zona pellucida present. Embryo progresses from morula to the blastocyst. Early evidence of the blastocoelic cavity. In the blastocyst stage (zona-intact) there is a distinct inner cell mass and an outer layer of trophectoderm cells. Usually located in the uterine lumen.

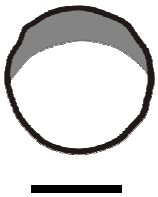
Embryonic age = 3 dpc (range 2-4 dpc)
Scale bars 50μm



Theiler Stage 05

Blastocyst (zona-free)
Zona free blastocyst. Invariably located within the uterine lumen.

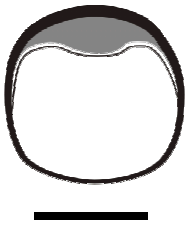
Embryonic age = 4 dpc (range 3-5.5 dpc)
Scale bar 100μm



Theiler Stage 06

Attachment of blastocyst
Blastocyst implants, first evidence of embryonic endoderm cells covering the blastocoelic surface of the inner cell mass.

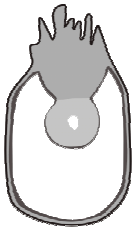
Embryonic age = 4.5 dpc (range 4-5.5 dpc)
Equivalent Witschi Stage in rat = 8
Equivalent Carnegie Stage in human = 4
Scale bar 100μm

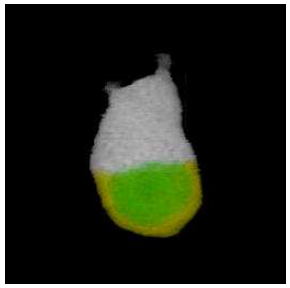


Theiler Stage 07

Implantation and formation of egg cylinder
Ectoplacental cone appears. Rapid increase in the number of inner cell mass cells leading to the formation of the epiblast with subsequent growth to form the egg cylinder. The proximal or visceral cells (opposite side from the trophoblastic cap) are cuboidal in shape. Primary endoderm lines the mural trophectoderm.

Embryonic age = 5 dpc (range 4.5-6 dpc)
Equivalent Witschi Stage in rat = 10
Equivalent Carnegie Stage in human = 5
Scale bar 100μm



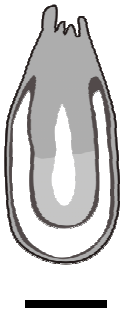


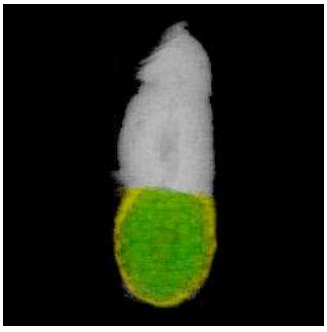
The TS07 EMAP model with the epiblast shown in green and primitive endoderm in yellow.

Theiler Stage 08

Differentiation of egg cylinder
Implantation site 2x3mm. The maternal tissue is invaded by trophoblast (primary) giant cells and the ectoplacental cone is invaded by maternal blood. Differentiation of the egg cylinder into embryonic and extra-embryonic regions and the formation of the pro-amniotic cavity. Reichert's membrane, which is non-cellular and secreted by the distal endoderm, first appears.

Embryonic age = 6 dpc (range 5-6.5 dpc)
Equivalent Witschi Stage in rat = 10-11
Equivalent Carnegie Stage in human = 5
Scale bar 100μm



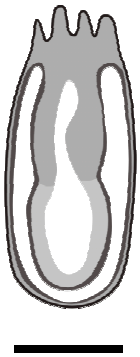


The TS08 EMAP model with epiblast shown in green and visceral endoderm in yellow.

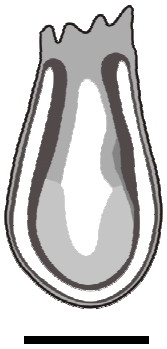
Theiler Stage 09

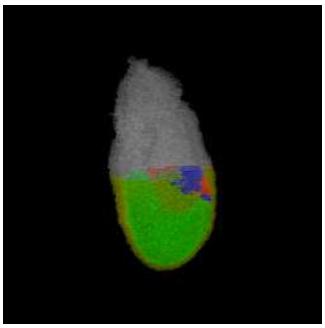
Advanced Endometrial Reaction

Stage 9a
Advanced egg-cylinder stage with the first evidence of an embryonic axis. There is clear morphological distinction between the embryonic and extra-embryonic ectoderm. The ectoplacental cone is further invaded by maternal blood and the original lumen of the uterine crypt has disappeared.
Equivalent Downs & Davies Stage : PS (pre-streak)
Scale bar 150μm



Stage 9b
Late in this stage gastrulation begins, producing the first mesodermal cells:
Equivalent Downs & Davies Stage : ES (early streak)
Scale bar 150μm





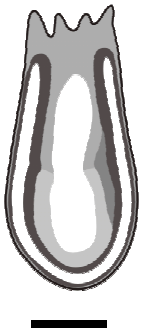
The TS09 EMAP model with epiblast shown in green, embryonic visceral endoderm in yellow, embryonic mesoderm in blue and primitive streak in red.

Posterior is shown to the right
Embryonic age = 6.5 dpc (range 6.25-7.25)
Equivalent Witschi Stage in Rat = 11
Equivalent Carnegie Stage in human = 6-7

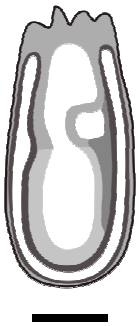
Theiler Stage 10

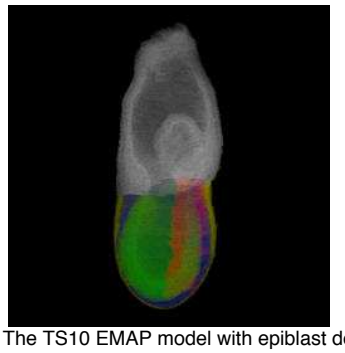
Amnion

Stage 10a
Tissue at the posterior end of the primitive streak bulges into the pro-amniotic cavity and forms the amniotic fold:
Equivalent Downs and Davies stage: MS (mid-streak)
Scale bar 150μm



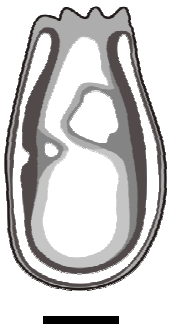
Stage 10b
In the mesoderm of the posterior amniotic fold small cavities coalesce to form a single cavity, the exocoelom
Scale bar 150μm





The TS10 EMAP model with epiblast depicted in green, embryonic mesoderm in blue, primitive streak in red and embryonic visceral endoderm in yellow.

Stage 10c
The allantoic bud first appears, gastrulation continues and the node becomes visible.
Scale bar 150μm



Embryonic age = 7.0 dpc (range 6.5-7.5 dpc)
Equivalent Downs & Davies stages: MS - LS (mid-streak to late streak)
Equivalent Witschi Stage in rat = 12
Equivalent Carnegie Stage in humans = 8

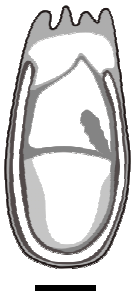
Theiler Stage 11

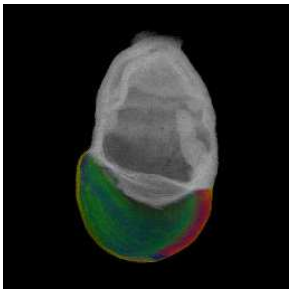
Neural Plate, Presomite stage

Stage 11a
The amniotic cavity is now sealed off into three distinct cavities - the amniotic cavity, the exocoelom and the ectoplacental cleft. The neural plate is defined anteriorly and the head process is developing. In the midline, subjacent to the neural groove, the notochodal plate is visible. Scale bar 200μm

Stage 11b
The allantoic bud elongates. Scale bar 200μm

Stage 11c
The rostral part of the neural plate begins to enlarge to form the head folds. The neural groove is visible. Scale bar 200μm





The TS11 EMAP model showing epiblast in green, embryonic mesoderm in blue, primitive streak in red and embryonic visceral endoderm in yellow.

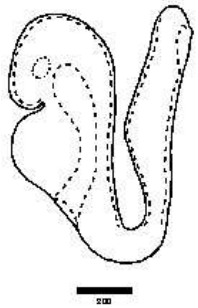
Stage 11d
Head folds continue to enlarge and the foregut pocket begins to form. Scale bar 200μm

Embryonic age = 7.5 dpc (range 7.25-8 dpc)
Equivalent Downs & Davies stages: OB-EB (no allantoic bud to early allantoic bud); LB-EHF-LHF (late allantoic bud to early head fold to late head fold)
Equivalent Witschi Stage in rat = 12-13. Equivalent Carnegie Stage in humans = 9

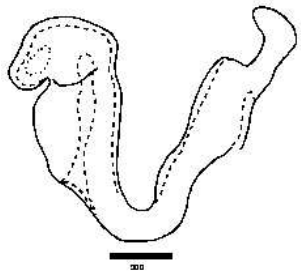
Theiler Stage 12

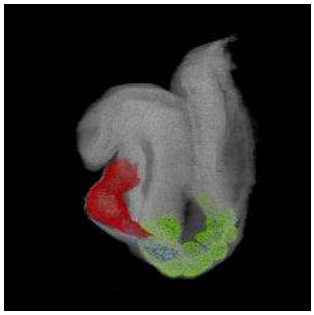
First Somites
Unturned embryo with first appearance of somite pairs

Stage 12a
1-4 somites. The allantois extends further into the exocoelom and the maxillary components of the 1st branchial arch become prominent. The preotic sulcus is visible in the 2-3 somite embryo. The cardiogenic plate begins to form and the foregut pocket is clearly visible.
Scale bar 200μm



Stage 12b
5-7 somites. The headfolds are particularly prominent and neural closure occurs in the region of the 4th and 5th somites, extending in both directions from this site. The optic placodes are first evident and become indented to form the optic pits. The heart rudiment develops rapidly. The allantois contacts the chorion at the end of this stage.
Absent: The 2nd branchial arch and >7 somites.
Scale bar 300μm





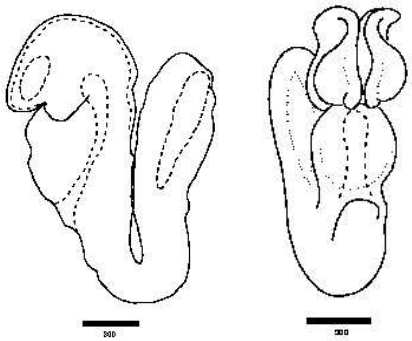
The TS12 EMAP embryo showing the outflow tract, primitive ventricle and atrium in red, sinus venous in blue and somites in green

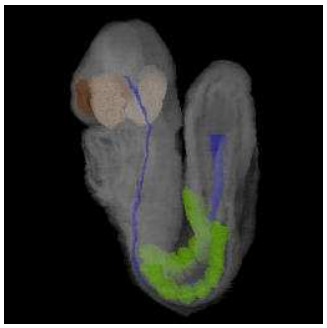
Embryonic age = 8 dpc (range 7.5-8.75 dpc)
1-7 somite pairs
Equivalent Witschi Stage in rat = 14-15
Equivalent Carnegie Stage in humans = 9

Theiler Stage 13

Turning of the embryo

This is a short period with turning initiated in embryos with 6-8 pairs of somites and usually completed in embryos with 14-16 pairs of somites. The first branchial arch has maxillary and mandibular components but the maxillary process is not visible until later (TS16). A second branchial arch is now evident. There is evidence of regionalisation of the heart and the neural tube is closed from a point opposite the outflow tract to the proximal part of the tail. Absent: 3rd branchial arch. Scale bars 300μm





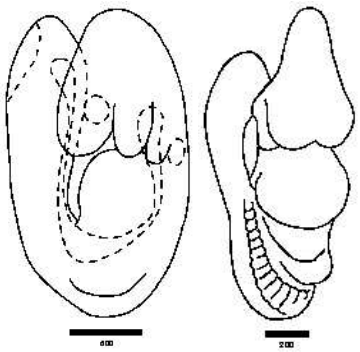
The EMAP TS13 embryo with notochord in blue, somites in green, branchiall arches in pink and optic vesicle in brown.

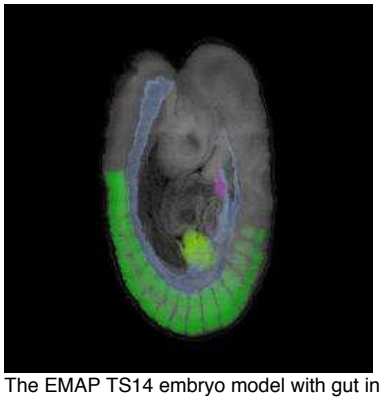
Embryonic age = 8.5 dpc (range 8-9.25 dpc)
8-12 somite pairs
Equivalent Witschi Stage in rat = 15
Equivalent Carnegie Stage in humans = 10

Theiler Stage 14

Formation and closure of anterior neuropore

The rostral extremity of the neural tube closes in embryos with usually about 15-18 somite pairs and defines this stage. The otic pit becomes progressively more indented but not closed, the mandibular process of the 1st branchial arch is clearly visible. The 3rd branchial arch becomes visible late in the stage. An increasingly prominent ridge on the lateral body wall, approximately at the level of the 8th-12th somite, indicates the site of the future forelimb bud. Absent: forelimb bud. Scale bars 500μm and 200μm





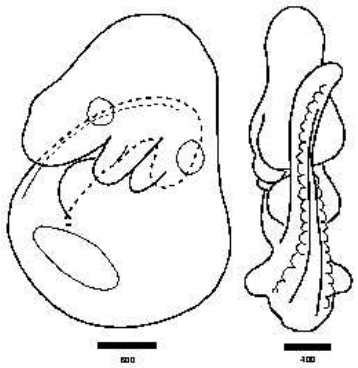
The EMAP TS14 embryo model with gut in blue, thyroid primordium in pink, septum transversum in lime green and somites in green.

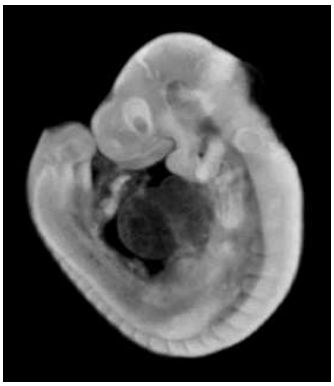
Embryonic age = 9 dpc (range 8.5-9.75 dpc)
13-20 somite pairs
Equivalent Witschi Stage in rat = 16
Equivalent Carnegie Stage in humans = 11

Theiler Stage 15

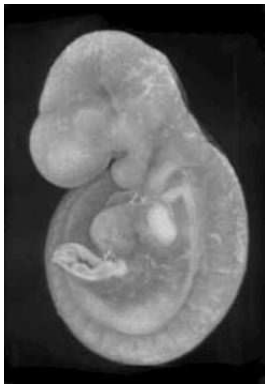
Formation of posterior neuropore, forelimb bud

The posterior neuropore forms and the condensation of the forelimb bud becomes apparent near the 8th-12th somite pairs. A distinct condensation of the hind limb bud appears just at the end of the stage. The forebrain vesicle subdivides into telencephalic and diencephalic vesicles.
Absent: hindlimb bud, Rathke's pouch.
Scale bars 500μm and 400μm





The early TS15 EMAP model



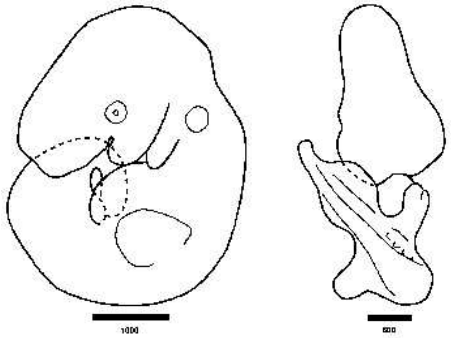
The later TS15 EMAP model

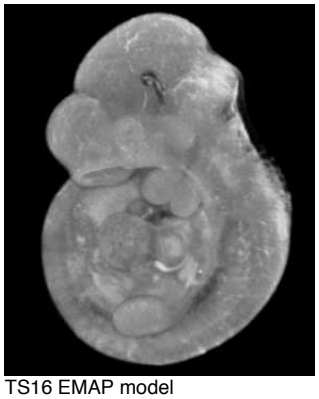
Embryonic age = 9.5 dpc (range 9-10.25 dpc)
21-29 somite pairs
Equivalent Witschi Stage in rat = 17-19
Equivalent Carnegie Stage in humans = 12

Theiler Stage 16

Closure of posterior neuropore. Hind limb bud and tail bud

The hind limb bud becomes visible at the level of the 23rd-28th somites. The tail bud appears as a short stump and the 3rd and 4th branchial arches are distinctly concave. Rathke's pouch and the nasal processes start to form. At the end of this stage the posterior neuropore begins to close. Absent: thin and long tail. Scale bars 1000μm and 500μm





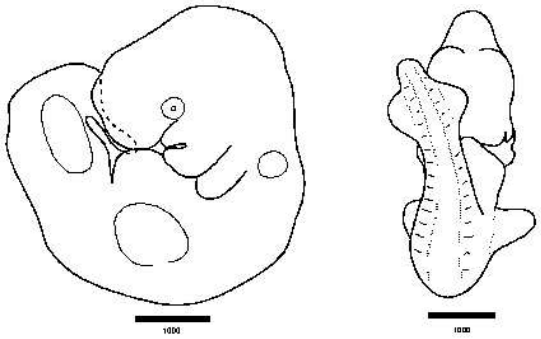
TS16 EMAP model

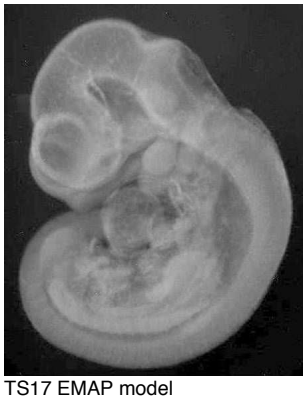
Embryonic age = 10 dpc (range 9.5-10.75 dpc)
30-34 somite pairs
Equivalent Witschi Stage in rat = 20-21
Equivalent Carnegie Stage in humans = 13-15

Theiler Stage 17

Deep Lens Indentation

The most obvious distinguishing features are the deepening of the lens pit, with a narrowing of its outer pore-like opening, and the first appearance of the physiological umbilical hernia. The 1st branchial arch is conspicuously divided into maxillary and mandibular components. There is advanced development of the brain tube and the tail elongates and thins. Absent: nasal pits.
Scale bars 1000μm



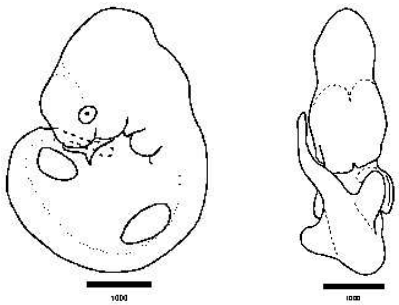

TS17 EMAP model

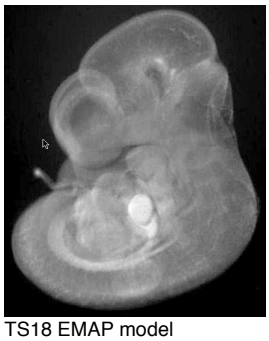
Embryonic age = 10.5 dpc (range 10-11.25 dpc)
35-39 somite pairs
Equivalent Witschi Stage in rat = 24-25
Equivalent Carnegie Stage in humans = 13-15

Theiler Stage 18

Closure of Lens Vesicle

The primary externally recognisable feature is the progressive closure of the lens vesicle. The somites in the cervical region are no longer visible and the rapid growth of the brain is striking. The nasal pits start to form. Absent: auditory hillocks, anterior footplate.
Scale bars 1000μm



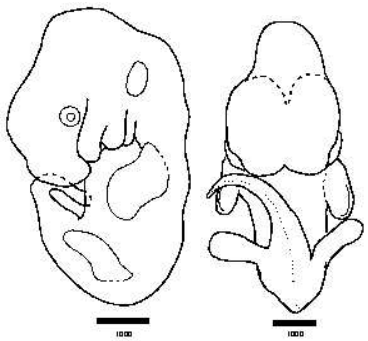

TS18 EMAP model

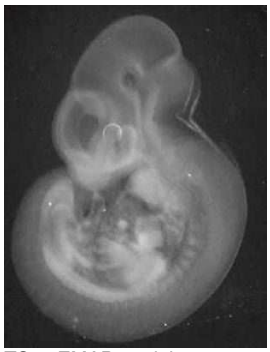
Embryonic age = 11 dpc (range 10.5-11.25 dpc)
40-44 somite pairs
Equivalent Witschi Stage in rat = 25-26
Equivalent Carnegie Stage in humans = 13-15

Theiler Stage 19

Lens vesicle completely separated from surface

The lens vesicle becomes completely closed and detached from the ectoderm. The peripheral margins of the eye become well defined. The forelimbs are seen to be divided into two regions, the proximal part consisting of the future limb-girdle and 'arm' and the more peripheral part which forms a circular or paddle-shaped 'handplate' (anterior footplate). The medial and lateral margins of the otic pit are coming together reducing the entrance to a narrow slit and the auditory hillocks become visible.
Absent: retinal pigmentation, signs of 'fingers'. Scale bars 1000μm





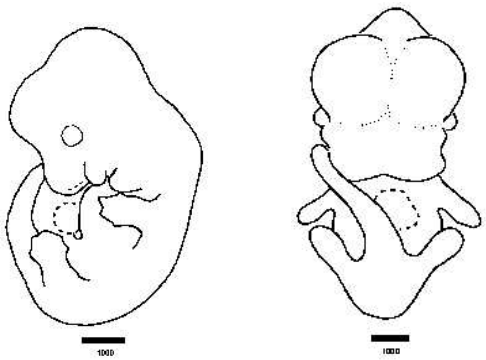
TS19 EMAP model

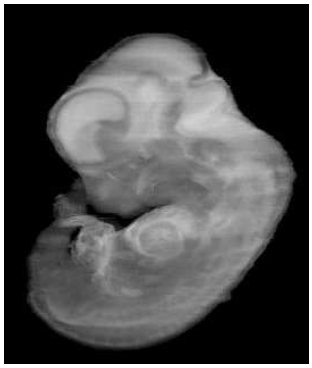
Embryonic age = 11.5 dpc (range 11-12.25 dpc)
45-47 somite pairs
Equivalent Witschi Stage in rat = 26-27
Equivalent Carnegie Stage in humans = 16

Theiler Stage 20

Earliest signs of fingers

The 'handplate' (anterior footplate) is no longer circular but develops angles which correspond to the future digits. The posterior footplate is also distinguishable from the lower part of the leg. It is possible to see the pigmentation of the pigmented layer of the retina through the transparent cornea. The tongue and brain vesicles are clearly visible.
Absent:5 rows of whiskers, indented handplate. Scale bars 1000μm





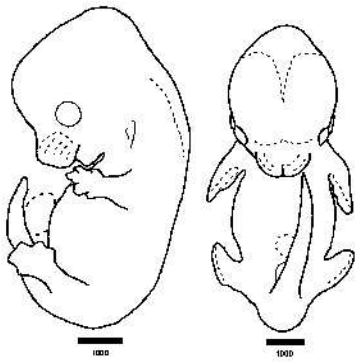
TS20 EMAP model

Embryonic age = 12 dpc (range 11.5-13 dpc)
48-51 somite pairs
Equivalent Witschi Stage in rat = 28
Equivalent Carnegie Stage in humans = 17

Theiler Stage 21

Anterior footplate indented, marked pinna

The distal borders of the anterior and posterior footplates are now indented and the digit widths and locations can be discerned. The 'elbow' and 'wrist' are now identifiable. The pinna rapidly develops and forms a crest at right angles to the head. Five rows of vibrissae are visible as well as a prominant hair follicle over the eye and another over the ear. The lens vesicle has lost its lumen. The physiological umbilical hernia is prominent. Absent: hair follicles, distally separate fingers.
Scale bars 1000μm

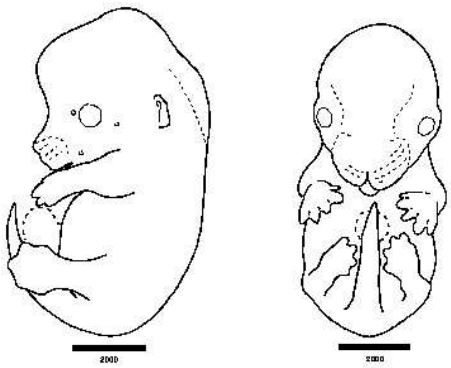


Embryonic age = 13 dpc (range 12.5-14)
52-55 somite pairs
Equivalent Witschi Stage in rat = 29-30
Equivalent Carnegie Stage in humans = 18-19

Theiler Stage 22

Fingers separate distally

Individual 'fingers' are visible in the anterior footplate and there are deep indentations between the 'toes' which are not yet separated. The long bones of the limbs are present and there are hair follicles in the pectoral, pelvic and trunk regions. The pinna is turned forwards and the umbilical hernia is conspicuous. Absent: hair follicles in the cephalic region. Scale bars 2000μm

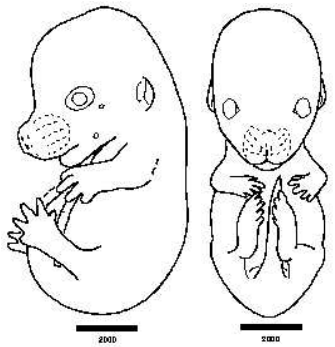


Embryonic age = 14 dpc (range 13.5-15 dpc)
56-~60 somite pairs
Equivalent Witschi Stage in rat = 31
Equivalent Carnegie Stage in humans = 20-23

Theiler Stage 23

Toes separate

The 'toes' separate and are clearly divergent, not becoming parallel until later. Hair follicles are present in the cephalic region but not at the periphery of the vibrissae. The pinna covers more than half of the external auditory meatus and the eyelids are still open.
Absent: nail primordia, 'fingers' 2-5 parallel.
Scale bars 2000μm

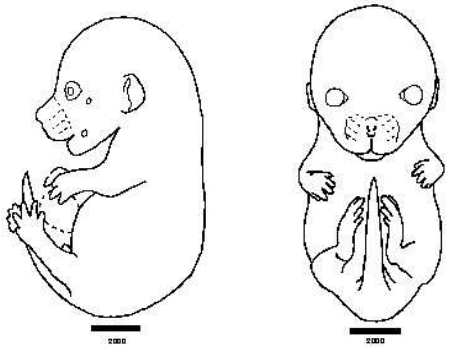


Embryonic age = 15 dpc
>60 somite pairs
Equivalent Witschi Stage in rat = 32
Human foetal period.

Theiler Stage 24

Reposition of umbilical hernia

'Fingers' 2-5 are nearly parallel. Nail primordia are visible on the 'toes'. The eyelids have fused in most cases by the end of the stage and the pinna almost completely covers the external auditory meatus. The umbilical hernia is disappearing and there is a corresponding increase in the size of the peritoneal sac.
Absent: 'fingers' and 'toes' joined together.
Scale bars 2000μm

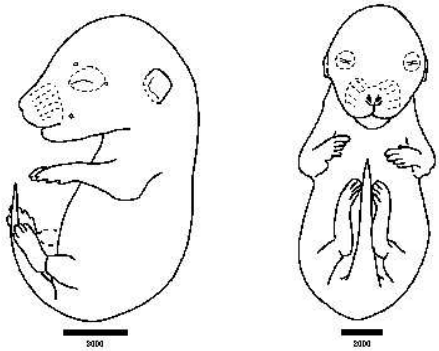


Embryonic age = 16 dpc
> 60 Somite pairs
Equivalent Witschi Stage in rat = 33

Theiler Stage 25

Skin wrinkled

The skin has thickened and formed wrinkles and the subcutaneous veins are less visible. The 'fingers' and 'toes' have become parallel and the umbilical hernia has disappeared. The eyelids have fused. Whiskers are just visible.
Absent:ear extending over auditory meatus, long whiskers.
Scale bars 3000μm and 2000μm

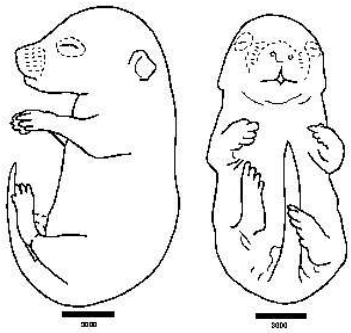


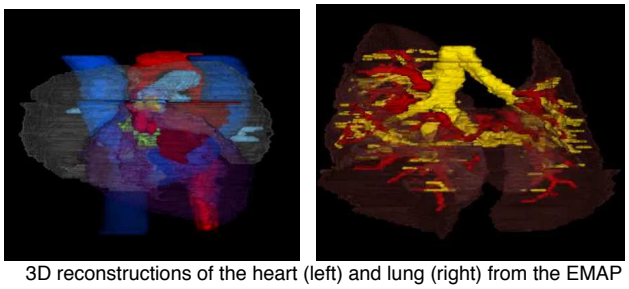
Embryonic age = 17 dpc
Equivalent Witschi Stage in rat = 34

Theiler Stage 26

Long whiskers

The whiskers that were present at stage 25 are definitely longer and the skin has thickened. The pinna is larger and such that virtually none of the lumen of the auditory meatus is visible. The eyes are barely visible through the closed eyelids.
Scale bars 3000μm





3D reconstructions of the heart (left) and lung (right) from the EMAP TS26 model.

Embryonic age = 18 dpc
Equivalent Witschi Stage in rat = 35

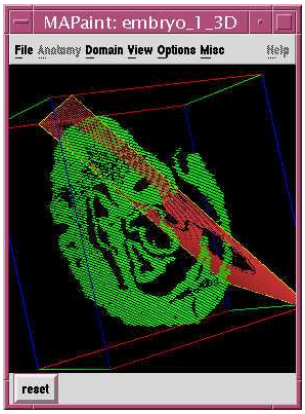
APPENDIX II: MAPaint

This program is ONLY available on the CD-ROM version of the Atlas and not online through the website. MAPaint only runs on computers with a UNIX operating system e.g. SUN workstations; Mac OSX and PCs running Linux (see Appendix IV for more information on operating system requirements). For the purposes of this course, we have installed MAPaint on the computers. To do this yourself in your home laboratory, please refer to the "How To Install" instructions that are contained on the CD in the appropriate folder for your operating system.

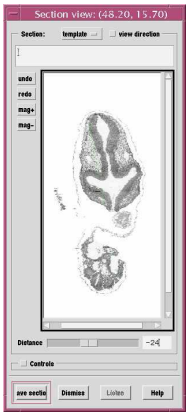
MAPaint has been written at EMAP for visualising and manipulating 3D data associated with the embryo models. It offers an interface to navigate through the 3D embryo models, taking sections in ANY plane to reveal histological detail. It allows you to transfer data into the 3D space of the embryo models (e.g. domains of gene expression, apoptosis, cell division etc). And once defined, it allows a 3D domain (e.g. anatomy, gene expression, apoptosis, cell division etc) to be visualised both in 3D space and on virtual sections cut from an embryo model.

For the TS07-TS14,TS20 and TS26 models (i.e. those reconstructed from serial sections with painted anatomical domains), you can identify tissues within ANY section you choose.

MAPaint will allow you to take virtual sections in ANY plane through the 3D embryo models to reveal internal structure:

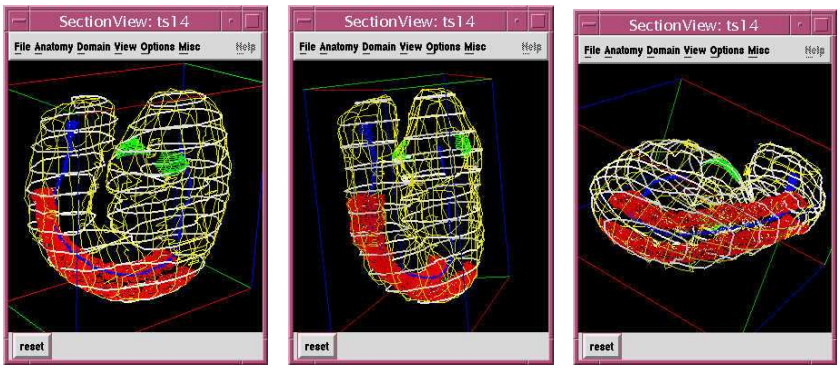


3D embryo showing section plane



Selected section

Various anatomical domains may be selected and viewed in 3D space:



Somites (red), notochord (blue) and surface ectoderm over the eye (green) displayed in 3D in the TS14 model.

Full instructions on how to use MAPaint are avaiable on the EMAP CD-ROM which contains the MAPaint program itself.

APPENDIX III: Computing Requirements

Recommended web browsers for use with the EMAP website and associated Java applets.

PC users …

Windows 95, 98, 2000 - use Internet Explorer version 5 (or greater).

Mac users…

MacOS9 - use Internet Explorer version 5 (or greater)

MacOSX – use Internet Explorer version 5.1 (or greater)

UNIX users…

Solaris (Sun or PC), IRX (SGI Indigo) or Linux (PC) – use Netscape version 4 (or greater)

A comprehensive list of other combinations of Operating Systems and Web Browsers and their compatibilities with the Atlas viewing programs can be found at…

http://genex.mrc.ac.uk/CDROM_online/macd/sysReq.html

Operating system requirements for running MAPaint

MAPaint requires a UNIX operating environment

PC users MAPaint will operate on a PC only if it uses the Linux operating environment. It has been tested and will operate on RedHatLinux. It will not run on a PC operating Windows.

Mac users MAPaint will operate on OSX only. It will not run on OS9 or earlier operating systems.

UNIX users MAPaint has been tested and will run on Solaris2.6, Solaris2.8, RedHatLinux, IRIX6.

MAPaint can be installed from the EMAP Atlas CD-ROM. Installation instructions for each of the above system can be found on the CD.

Operating system requirements for the EMAGE interface

The EMAGE interface requires Java v.1.4 (or JRE2 v1.4 (Java Runtime Environment 2, version 1.4)) and JavaWebStart 1.2 on you computer.

APPENDIX IV: References

Downs, K.M. and Davies, T. **(1993)** *Staging of gastrulating mouse embryos by morphological landmarks in the dissecting microscope* Development 118:1255-1266.

Kaufman, M. *The Atlas of Mouse Development*. Academic Press, London, **1992.**

Sharpe, J., Ahlgren, U., Perry, P., Hill, B., Ross,A., Hecksher-Sorensen, J., Baldock, R. and Davidson, D. **(2002)** *Optical Projection Tomography as a Tool for 3D Microscopy and Gene Expression Studies* Science. 296: 541-545.

Theiler, K. *The House Mouse - Atlas of Embryonic Development*. Springer-Verlag, New York, **1989.**

Wilkinson, D.G. (ed) *In Situ Hybridisation - A Practical Approach*. (2nd ed.) Oxford Univeristy Press, Oxford, **1998.**

APPENDIX V: Glossary

Carnegie Staging	Staging system for human embryo (see http://anatomy.med.unsw.edu.au/cbl/embryo/wwwhuman/Stages/CStages.htm)
DDBJ	DNA Data Bank of Japan http://www.ddbj.nig.ac.jp/Welcome.html
EMAP	Edinburgh Mouse Atlas Project http://genex.hgu.mrc.ac.uk/
EMAGE	Edinburgh Mouse Atlas of Gene Expression http://genex.hgu.mrc.ac.uk/
EMBL	European Molecular Biology Laboratory http://www.embl-heidelberg.de/
EmbryoView	Java 3D Views and MPEG movies of EMAP embryo models
IMAGE	Integrated Molecular Analysis of Genomes and their Expression Consortium http://image.llnl.gov/
Java	A computer language
Java Web Start	A Java application which runs the EMAP Anatomy and EMAGE databases
MGEIR	Mouse Gene Expression Information Resource (EMAGE + GXD)
GXD	Gene Expression Database, Jackson Laboratory, USA http://www.informatics.jax.org/mgihome/GXD/aboutGXD.shtml
GO	Gene Ontology Consortium http://www.geneontology.org/
MAPaint	Program to section 3D EMAP models in any plane

MGI	Mouse Genome Informatics, Jackson Laboratory, USA http://www.informatics.jax.org/mgihome/
NCBI	National Center for Biotechnology Information, USA http://www.ncbi.nlm.nih.gov/
GenBank	Sequence Database at NCBI http://www.ncbi.nlm.nih.gov/Genbank/index.html
Ontology	A controlled descriptive vocabulary
OPT	Optical Projection Tomography - a 3D visualisation technique.
Paintbrush	Program used to paint gene expression patterns within the EMAGE interface
PubMed	Database of Medical Literature, National Library of Medicine, USA. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi
PMID	PubMed Identifier Number
RIKEN	The Institute of Physical and Chemical Research, Japan. http://www.riken.go.jp/
Section Browser	Java program to choose pre-selected section from EMAP models and identify anatomical structures within them
Section Movies	MPEG format movies of every virtual section taken through the EMAP models in the transverse, sagittal and frontal planes
Slice Chooser	Java program to select sections in any plane from an EMAP 3D embryo model
Theiler Staging	Staging system for mouse embryos
UniGene	Experimental system for partitioning GenBank sequences into a non-redundant set of gene-orientated clusters. http://www.ncbi.nlm.nih.gov/UniGene/
UNIX	Computer operating system
Witschi Staging	Staging system for rat embryos
xterm	Window used in UNIX environment to enter commands